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Devoted to the Advancement of the Biological Sciences with Special Reference to the Factors of Evolution

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A STUDY OF EVOLUTIONARY CHROMOSOME CHANGES IN SCIARA (DIPTERA). CHROMOSOME C IN THE SALIVARY GLAND CELLS OF SCIARA OCELLARIS AND SCIARA REYNOLDSI

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INTRODUCTION

WITHIN recent years numerous studies have been made on the relation of chromosomal aberrations to the problems of evolution and speciation. In these studies, particularly in Drosophila, two lines of approach have been followed. One has involved the investigation of chromosomal relationships between various geographical populations within a species, the other a comparison of conditions in different species, especially through study of species hybrids. The evidence derived from these sources has indicated the type and frequency of chromosome changes which are found in nature and which consequently may have evolutionary significance. In Drosophila, where there is considerable uniformity of evidence, tentative generalizations can be made as to the mode of evolution in this genus. It is not known, however, whether conditions in Drosophila are characteristic of other organisms. This can only be determined by comparative studies. Such studies can best be carried out on other Diptera where the giant salivary gland chromosomes can be used. Some studies of this kind, although

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not extensive, have been made on two other genera, Sciara and Chironomus, both of which belong to the lower Diptera and thus are taxonomically far removed from Drosophila. The present paper is concerned with a study of the evolutionary changes which have occurred in one particular chromosome during the divergence of two closely related species of Sciara. The evidence is based primarily on a study of the salivary gland chromosomes in hybrids between the two species, *Sciara ocellaris* Comst., and *S. reynoldsi* Metz. Earlier work on these species (Metz, 1938*a*, and Metz and Lawrence, 1938) has shown that chromosome *C* presents certain peculiarities which necessitate making it the object of a special study. The peculiarities will be pointed out below. As in the case of the earlier work just mentioned the broader purpose of the present study is to compare conditions in Sciara with those observed by other investigators in Drosophila.

Conditions in Drosophila have been reviewed by Dobzhansky (1941) and will only be summarized here. The evidence indicates that the most frequent microscopically visible chromosome changes accompanying speciation in this genus are gross rearrangements, including inversions and translocations, especially the former. In contrast to the relatively high frequency of these gross rearrangements there appears to be a very low frequency of minute modifications and among these deficiencies and duplications are particularly rare. In Sciara, on the other hand, the preliminary observations on the species hybrids mentioned above indicate that in this material the reverse is true. Minute modifications are relatively common and gross rearrangements are rare. In the present study gross rearrangements have been found in chromosome *C*, thus making the conditions in the present material less different from those in Drosophila than the earlier evidence seemed to indicate. Similar results have recently been obtained by Carson (1944) and McCarthy (1945) on other species of Sciara. The existing differences are in the relative frequency with which the various

types of chromosome rearrangements are observed rather than in the types of rearrangements themselves.

The present study deals with changes in the chromosome designated chromosome C. The study is primarily concerned with the alterations which have taken place in this chromosome during the evolution of the two species as revealed by study of the hybrids. In addition, supplementary observations are included on spontaneous changes found within different strains of the same species. Chromosome *C* presents certain peculiarities which

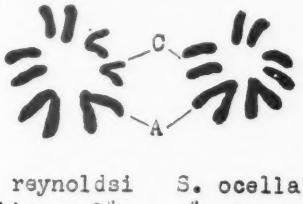

FIG. 1

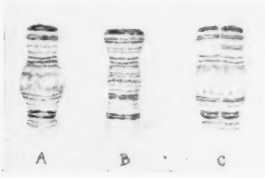

FIG. 2

FIG. 1. Metaphase figures from larval gonads. FIG. 2. A. End 2 of *S. ocellaris* to show the "bulb." B. The same end showing the region of the "bulb" in the banded condition. The "bulb" occupies the region of three bands. C. End 2 of the "hammond" stock. The left homologue shows a deficiency of two bands. In the heterozygous condition this deficiency often causes asynapsis of the ends of the chromosome.

are not noted in chromosomes *A*, *B* and *X*. The main peculiarity is the tendency this chromosome has in *Sciara reynoldsi* to separate transversely into two approximately equal segments during the development of the salivary gland cells (Metz, 1940). This regular separation has made it impossible to determine the actual sequence of parts in the intact germ-line chromosome and thus to compare chromosome *C* accurately with its homologue in *S. ocellaris*. As will be brought out below, the present study offers two possibilities concerning the origin of the differences between chromosome *C* in *S. reynoldsi* and the corresponding chromosome in *S. ocellaris*. In addi-

tion a spontaneous inversion detected in chromosome *C* of *S. ocellaris* will be described.[2]

Methods and Materials

Metz (1931, 1938*a*) has pointed out that in the genus Sciara several different types of sex ratios are found.

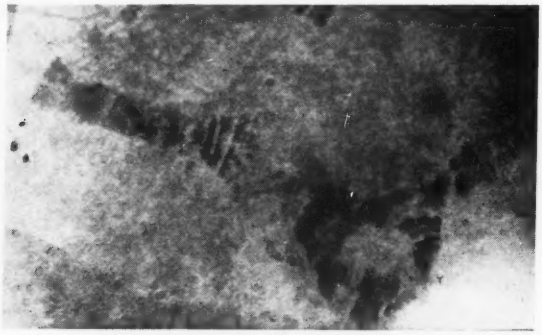

Fig. 3

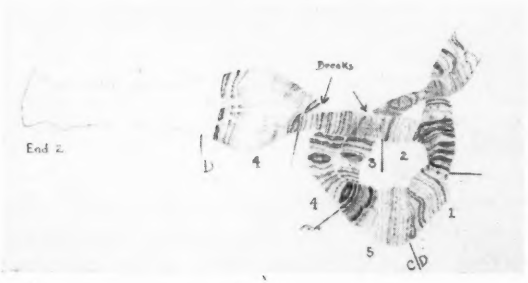

Fig. 4

Fig. 3. Photomicrograph of the inversion occurring in the "Reeve" stock (*S. ocellaris*). The inversion is near *end 2* of chromosome *C*. Fig. 4. Camera lucida drawing of region shown in Fig. 3 to show details of banding in the inversion figure.

In some species pair matings give unisexual families, *i.e.*, families in which the individuals are all or nearly all of one sex, either males or females; in other species bisexual

[2] This work was done at the University of Pennsylvania under the direction of Professor C. W. Metz. I wish to thank him for suggesting the problem and for many helpful suggestions during the course of the investigation.

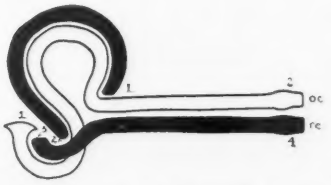

FIG. 5. Diagram to show the double-looped figure which would be produced by the ocellaris-reynoldsi homologues if complete pairing occurred between all homologous regions.

families are regularly produced, *i.e.*, families in which the individuals are males and females in more or less equal numbers; in still other species both types of sex ratios are found. The stocks of *S. ocellaris* used in this

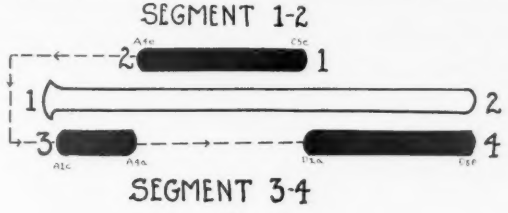

FIG. 6

investigation produce unisexual families and the stocks of *S. reynoldsi* produce bisexual families.

Sciara ocellaris is widely distributed throughout the United States, but *Sciara reynoldsi* has been taken thus far at only one place in Alabama and two places in North Carolina. The *S. ocellaris* stocks from which the indi-

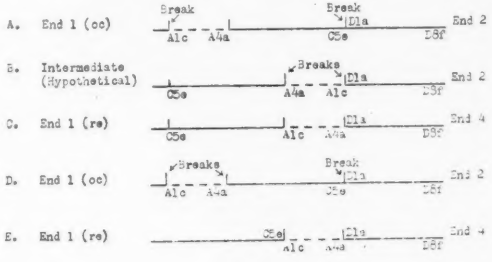

FIG. 7

viduals for this investigation were secured came originally from the following locations. "Berry" and "Reeve" stocks were collected in greenhouses in Alabama; "Hammond" was collected in Virginia. The four stocks of *S. reynoldsi* used in this study are designated as 379, 598, 600 and "yellow." Intraspecific crosses were made between the three stocks of *S. ocellaris* and between the four stocks of *S. reynoldsi*. The interspecific crosses between *S. ocellaris* and *S. reynoldsi* involved the use of *ocellaris* females and *reynoldsi* males; the reciprocal mating is not fertile.

Permanent smears of the salivary gland cells were made using a staining technique similar to that of Hughes (1939) except that the slides were placed in an alcohol vapor chamber for two hours only.

The long maps of chromosome *C* of *S. ocellaris* and *S. reynoldsi* (Figs. 9 and 10) are composite camera lucida drawings made from favorable smears. The map of chromosome *C* of *S. ocellaris* is divided into four large sections (Fig. 9). Beginning at *end 1* these are lettered as AA, BB, CC and DD. Each section is subdivided into smaller regions 1, 2, 3, etc. Cross band regions are represented by small letters a, b, c, etc. The *S. reynoldsi* chromosome *C* (Fig. 10) has been lettered to show regions homologous with those of *S. ocellaris*. The drawings of the inversion (Fig. 4) and of the conditions in the hybrid (Figs. 12, 14, 17, 21 and 22) are likewise camera lucida

FIG. 8. Composite drawing of chromosome *C*, ocellaris-reynoldsi hybrid to show homologous regions. The ocellaris homologue is centrally located. Segment 1–2 (above the ocellaris homologue) represents one portion of the reynoldsi chromosome. This segment is inverted (see Fig. 10 and text for explanation). The other portion of the reynoldsi chromosome, segment 3–4 is arbitrarily shown in two parts. The portion *A 3 c–A 4 a* represents either an inversion or transposition. Small arrows indicate small differences described in text.

FIG. 9. Schematized camera lucida drawing of chromosome *C* of *Sciara ocellaris*.

FIG. 10. Schematized camera lucida drawing of chromosome *C* of *Sciara reynoldsi*. This chromosome is always observed in two segments.

FIG. 8

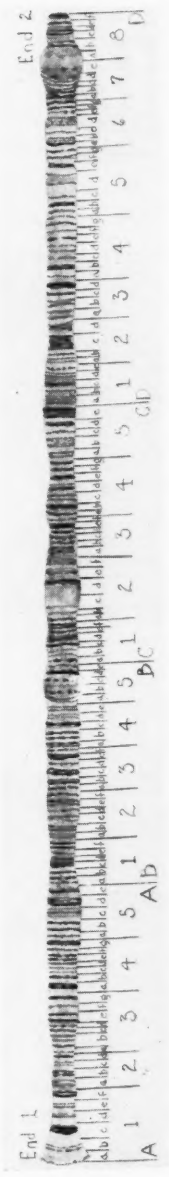

FIG. 9

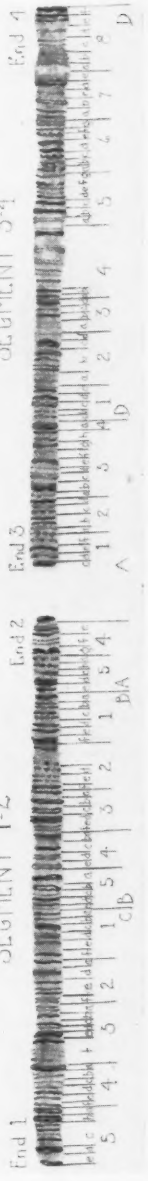

FIG. 10

drawings, but each is made from a single cell. All drawings were made with a 12 × ocular and 95 × oil immersion lens.

Basic Chromosome Group in Sciara ocellaris and Sciara reynoldsi

In the nuclei of the salivary gland cells of *S. ocellaris* and *S. reynoldsi* there are four chromosome pairs, *A, B, C*

Fig. 11

Fig. 12

Fig. 15

Figs. 11, 12, & 15 were all made from the same hybrid figure. Figure 15 shows the two homologues, reynoldsi (black) and ocellaris (white). Cross-hatched area represents the paired region and also the region shown in Fig. 11. This is the region which may represent an inversion. Note that *end 1* of the reynoldsi chromosome corresponds to a region lying within the ocellaris chromosome. Fig. 12 is a camera lucida drawing of region shown in Fig. 11. See also the diagram of this region in Fig. 8.

and *X*. In the larval germ-line at metaphase the chromosome complement of *S. reynoldsi* consists of two pairs of rods and two pairs of V-shaped chromosomes (Fig. 1), one pair of V's being approximately twice as large as the other; in ocellaris, however, the chromosome pair which is homologous to the smaller pair of V's may have a median or slightly subterminal spindle fiber attachment (Crouse, 1939). This is chromosome *C* in the nuclei of

FIG. 13

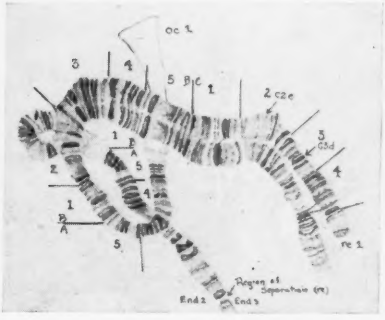

FIG. 14

FIG. 13. Photomicrograph of another hybrid preparation showing the relationship between segment 1–2 (reynoldsi) and the ocellaris chromosome. A small portion of *end 3* (reynoldsi segment 3–4) is visible.

FIG. 14. Camera lucida drawing of region shown in Fig. 13 to demonstrate details of banding.

the salivary gland cells (Crouse, 1943), the chromosome under consideration in this paper.

The fact that chromosome *C* is V-shaped in reynoldsi and rod or V-shaped in ocellaris is interesting, since chromosome *C* in reynoldsi tends to separate transversely into two approximately equal segments in the salivary gland cells (but not in ordinary mitotic cells).

A description will be presented of chromosome *C* in ocellaris and in reynoldsi and the homologies between

them in the hybrid will be pointed out, followed by an interpretation of the possible origin of the differences.

Chromosome C–Sciara ocellaris

The composite drawing of chromosome *C* (Fig. 9) has been schematized for the purpose of analysis. However, an attempt has been made to represent as accurately as

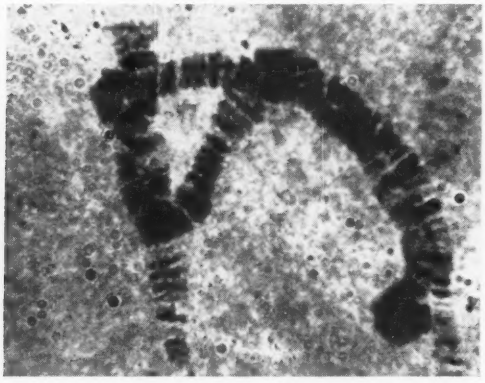

Fig. 16. Photomicrograph of same region shown in Figs. 11 and 13 but from a different preparation. Segment 1–2 (reynoldsi) and the ocellaris homologue are shown at the right of the figure. At the left is a portion of segment 3–4 (reynoldsi) and the corresponding region in ocellaris. The fan-shaped *end 1* of ocellaris is visible at the top of the figure. This paired region represents the transposition or small inversion described in the text. Figures showing even a partial picture of pairing between the two segments of reynoldsi and the ocellaris chromosome are rare.

possible characteristic regions and to differentiate the different stain intensities appearing among the several bands.

Chromosome *C* in the three stocks of ocellaris investigated is intermediate in length between *A* and *B*. With one exception, which will be pointed out later, the two homologues composing this chromosome pair show complete synapsis. This chromosome is readily distinguished by the characteristic fan-shaped appearance of one end,

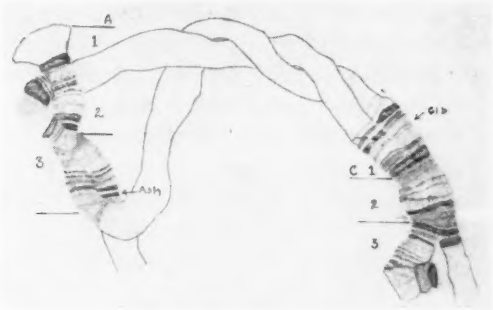

FIG. 17. Camera lucida drawing of regions shown in Fig. 16.

designated *end 1* (Fig. 9). The fan-shaped condition varies but little in the numerous preparations. The first band of *end 1, A 1 a,* appears as small irregular accumulations of deeply stained chromatic material. The broken

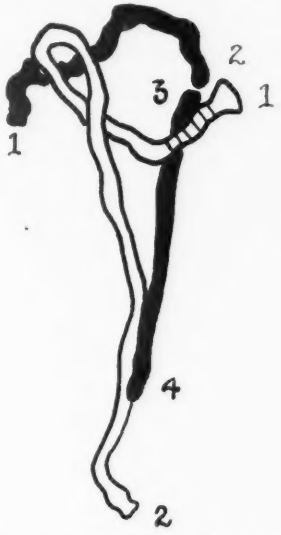

FIG. 18. Outline drawing of reynoldsi (black) and ocellaris (white) homologues shown in Fig. 19. Cross-hatched region indicates area of pairing; this is the region of the small inversion or transposition.

bands, *A 1 b*, occupying the median portion of the fan, are always evident although they stain differently in the various preparations. The appearance of region *A 1 c* is variable. It is usually seen as a large heavily stained capsule; at other times two heavily stained bands are discernible. In most preparations, *end 2* shows a distinct "bulb" within five bands of the end. In other instances, and less frequently, this end shows the "normal" banded pattern. An analysis of the region in the banded condition shows that the "bulb" occupies the region of three bands (Figs. 2 A and B). As Metz (1938*b*) has pointed out, a "bulb" usually occurs at a specific chromosome region and involves the same bands.

Aside from the peculiarities distinguishing the ends of this chromosome, there are other features or "landmarks" which should be noted. Conspicuous in many preparations is the region *B 5 a d* (Fig. 9), characterized by two heavily stained bands separated by a relatively wide area in which are seen two broken bands and a lightly stained single band. The broken bands often appear as four irregular accumulations of chromatic material. Critical inspection of this region revealed these chromatic bodies to be connected with one another by fine chromatic "threads." In some instances the chromatic material is evenly distributed and this broken condition is not visible. Another characteristic region is *C 2 c d*. *C 2 c* contains a very light band. This region is separated from the capsule-like structure at *C 2 d* by a bandless region. The width of the bandless region varies. The capsule at *C 2 d* is divided into two portions. Region *D 1 a* is a typical vesiculated structure. The bulb described previously is located at *D 7 c d e* and *8 a b*. This region is seldom observed in the banded condition.

On analyzing chromosome *C* in the intraspecific matings of ocellaris, little variation is found among the three stocks used. The homologues composing chromosome *C* are completely paired. The only difference noted is at *end 2* in the "Hammond" stock. In the "Reeve" and

"Berry" stocks at region *D 8 d* there are present two heavily stained irregular bands. Frequently this region in the "Hammond" stock shows scattered chromatic droplets with no evidence of banding and when this deficiency is present in the heterozygous condition, it often

FIG. 19. Photomicrograph of ocellaris-reynoldsi hybrid chromosome *C* to show configuration and pairing of the two homologues in the region of the small inversion or transposition. *End 1* of ocellaris, with the exception of the fan-shaped end pairs with *end 3–A 4 a* (reynoldsi segment 3–4).

produces asynapsis of the chromosome ends (Fig. 2 C).

Although only a few stocks of ocellaris have been analyzed, it seems probable that gross rearrangements do not occur with the same frequency as in Drosophila and Chironomus. The first spontaneous rearrangement,

an inversion, was noted in the heterozygous condition in several cultures of the "Reeve" stock. Whether this was present in the original stock or arose in the laboratory is unknown, but it has persisted for several years. This heterozygous inversion is shown in the photomicrograph and the accompanying camera lucida drawings (Figs. 3 and 4). Analysis of the inversion figures indicates that breaks occurred at *C 4 a* and *D 4 a* (Fig. 9) and the section *C 4 a–D 4 a* became inverted.

Chromosome C–Sciara reynoldsi

The chromosomes of *S. reynoldsi* are not as favorable for study as those of *S. ocellaris*. In this species the ends of the salivary gland chromosomes adhere to one another. There is no apparent sequence in which the various chromosome ends are attached. Different combinations are found. The chromosomes may all be associated to form an irregular structure; two or more may be involved in a ring formation; or they may form a chain. This tendency for the ends of the chromosomes to adhere to one another occurs in other members of the Diptera. However, in Drosophila this terminal adhesion is not of random occurrence, but the association tends to follow a regular pattern (Hinton and Sparrow, 1941). In Habropogon, the robber fly, the chromosome pairs are found associated with non-homologous pairs either by their ends or by their intercalary regions (Ribbands, 1941). This is true for all stages of the meiotic prophase. In the salivary gland cells of Chironomus there is an association between non-homologous chromatic regions in addition to an association between chromosome ends where no chromatic material is present (Ribbands, 1941). In *S. reynoldsi* the chromatic regions are the points of association and terminal adhesion has a greater frequency than lateral adhesion.

As mentioned before, chromosome *C* in reynoldsi regularly separates into two approximately equal segments in the salivary gland nuclei. The ends of these segments

are often associated, but the association is at random, and therefore, it is not known which two of the four ends are attached in the ordinary chromosomes as found in the germ-line. In other words, the sequence of the two segments in the intact mitotic chromosomes is not known.

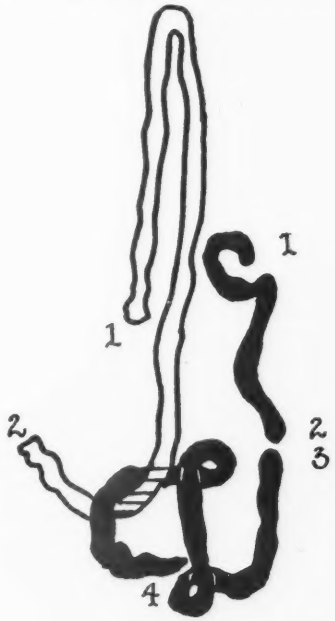

FIG. 20

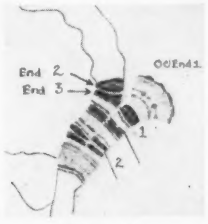

FIG. 21

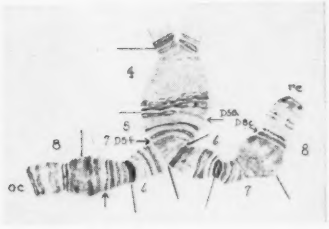

FIG. 22

FIG. 20. Outline of complete homologues, a portion of which is shown in Figs. 22 & 23. Cross-hatched region indicates region of pairing shown in Fig. 23.

FIG. 21. Camera lucida drawing of region shown in Fig. 19 to give details of banding. See also the diagram of this region in Fig. 8.

FIG. 22. Camera lucida drawing of paired region of Fig. 23 to show details of banding at *end 2* (ocellaris) and *end 4* (reynoldsi).

Nor are the evolutionary events responsible for this condition known. Later the homologous relationships between chromosome *C* in ocellaris and reynoldsi will be presented, and on the basis of these homologies, theoreti-

cal interpretations of possible evolutionary sequence will be given.

In the study of the pure stocks of reynoldsi and intraspecific crosses no small aberrations have been observed

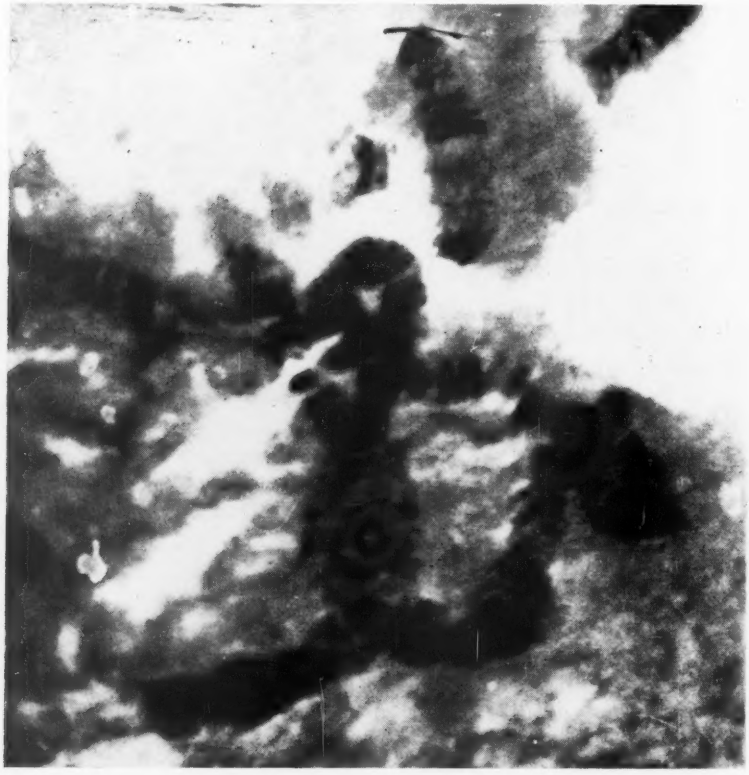

FIG. 23. Photomicrograph to show "normal" relationship between ocellaris-reynoldsi homologues and also to show similarity of banding pattern between the terminal portions of these homologues. Detail of this region is given in Fig. 22.

and in none of the crosses was any asynapsis encountered. The probable significance of the absence of the small differences will be noted later.

Structurally, chromosome *C* in reynoldsi shows some

characteristic "landmarks" which will help in the identification of particular regions. The region *C 5 e* (Fig. 10) at *end 1* is usually a dark irregularly shaped capsule, or it may appear as a thick heavily stained band. A slight constriction within five bands of this end helps to identify it. The other end of segment 1–2, *end 2*, is broad; the terminal band stains heavily and close to it is a structure which is composed of three dark bands, *A 4 e*. This is followed by two more lightly stained bands, two dark, and again two light and two dark, all equally spaced. At the termination of this series, there is a slight constriction. *End 4* of chromosome *C* in reynoldsi is similar to *end 2* of ocellaris, although the terminal band of the reynoldsi chromosome is more heavily stained. The other end of segment 3–4, *end 3*, shows no characteristics which are useful in its identification.

Chromosome C in ocellaris–reynoldsi hybrids

Preliminary observations on the salivary gland chromosomes of the hybrid were made by Metz and Lawrence (1938). They presented photomicrographs of the entire salivary gland complex. Their pictures are quite different from those seen in similar preparations of the pure strains of these species (Metz, 1935). Preparations of the latter show the four chromosomes with the two components of each closely paired so that their dual nature is not evident. In the hybrid, on the other hand, this dual nature is very evident and pairing occurs for short distances only. The ocellaris and reynoldsi components of each pair can be recognized with little difficulty. In all the preparations the ends of the reynoldsi chromosomes retain the tendency to adhere to one another.

In favorable preparations of the species hybrids, the *A*, *B* and *X* homologues of the two species lie more or less parallel to one another. The two homologues of a pair may coil around one another with partial pairing for short distances and differences in banding can readily be determined. In chromosome *C*, however, the figures are

complex (Figs. 11 through 23). Figs. 15, 18 and 20 are outline camera lucida drawings of the two homologues. These represent the simplest types of configurations observed. In each drawing a different paired region is represented (cross-hatched area). When these three regions are observed in the paired condition in one pair of homologues the figures are too complex to analyze (Metz, 1941). Diagrammatically they would form a double-looped figure (Fig. 5).

The relationship between the segments of the reynoldsi chromosome and the ocellaris homologue is shown in Fig. 6. The ocellaris chromosome is shown in outline and the two segments of the reynoldsi chromosome in solid black. Segment 1–2 (reynoldsi) pairs with the corresponding part of the ocellaris chromosome, but this part is intercalary—it does not include either end of the ocellaris chromosome. Segment 3–4 (reynoldsi) is arbitrarily shown in two parts to indicate homologies but is not actually broken in the material. The smaller portion of segment 3–4 pairs with the region near *end 1* of ocellaris. The larger portion of segment 3–4 corresponds, with the exception of a short region which will be discussed later, to the homologous part of the ocellaris chromosome.

Details of the relationships between the homologues in the hybrid are given in Figs. 11 through 23. Figs. 11 through 17 (photomicrographs and accompanying diagrams) demonstrate the relation between reynoldsi segment 1–2 and ocellaris region *A 4 e–C 5 e* (see also Fig. 8). Figs. 18, 19 and 21 show the pairing between the homologues at region *A 1 c–A 4 a*. The region of the break in the reynoldsi chromosome is indicated in Fig. 21. Region *D 5 b–end 4* (reynoldsi) pairs in a normal manner with the corresponding region in ocellaris (Figs 8, 20, 22 and 23). Actual synapsis between the homologues at region *D 1 a–D 5 a* has not been observed. Minute differences are indicated by small arrows and will be discussed below.

Knowing the homologies that exist in chromosome *C*

of ocellaris and reynoldsi we can speculate on the evolutionary events that may have occurred in deriving one species from another or the two from a common ancestor. For the sake of simplicity the present interpretations are based on the assumption that the apparently less widely distributed species, reynoldsi, has been derived from the more widely distributed one, ocellaris. The latter species thus becomes the "standard." In formulating our interpretations, we have first to select a particular sequence of the two segments of the mitotic chromosome in reynoldsi and then compare this sequence with the standard condition found in ocellaris. We have selected the sequence shown in Fig. 10 because it would involve the smallest number of changes in such an evolutionary transformation.

The differences between this reynoldsi chromosome and the ocellaris standard could have arisen by means of either (1) two inversions or (2) a single transposition. In the former interpretation (Figs. 7 A, B and C), it is assumed that breaks have occurred in the ocellaris chromosome at *A 1 c* and *C 5 e* (Fig. 7 A) and that this section became inverted. As a result the intermediate sequence shown in Fig. 7 B was produced. This primary inversion was followed by a smaller inversion within the original, with breaks occurring at *A 4 a* and *A 1 c* (Fig. 7 B) and a rotation of this section, giving the sequence of parts as observed in the reynoldsi chromosome (Fig. 7 C).

The alternative interpretation is that the modifications have been brought about by a single transposition as represented in Fig. 7 D and E. On this view the conditions found in the reynoldsi chromosome can be explained by assuming a single translocation of segment *A 1 c–A 4 a* from an original position near *end 1* (Fig. 7 D) as in ocellaris to a position adjacent to region *D 1 a* (Fig. 7 E). On this assumption segment 1–2 of the reynoldsi chromosome has the same orientation as the corresponding part of the ocellaris chromosome and has not undergone inversion.

It is not possible to choose between the alternative inversion and transposition hypotheses. The former is supported by the work of Carson (1944) and McCarthy (1945) who found numerous inversions in wild populations of several species of Sciara. On the other hand, the studies of Crouse (1939) suggest that transposition rather than inversion brought about the transformation of chromosome *C* within various strains of ocellaris from a rod-shaped chromosome to one with a median spindle fiber attachment.

Minute Modifications in ocellaris–reynoldsi Hybrids

Comparison of the two homologues in the species hybrids reveals many small differences in banding pattern. Some of these differences are obvious in the hybrid preparations where the two chromosomes are lying side by side; others are evident when the composite drawings of the homologues are compared. These differences may be exceedingly small, involving one or two bands, and usually do not interfere with pairing between the bands on either side, or they may involve larger sections of the chromosomes; in the latter cases pairing between the homologues is either not observed or pairing occurs but figures are confused and difficult to analyze. The small differences in banding are usually clear cut and can be demonstrated in the hybrid preparations. The larger differences are ascertained by comparing the homologues from pure strains of the two species. It is difficult to make accurate comparisons by the latter method because of the structural variability of individual bands and regions, and the inconstancy of pairing between homologous bands. For these reasons only the very obvious and consistently appearing differences will be noted here.

Throughout the hybrid preparations several regions are observed in which the bands are obscure. Such regions are evident at *D 4* (Figs. 22 and 23), and *C 2* (Figs. 11, 12, 13 and 14). Comparison of the ocellaris and reynoldsi homologues from "normal" individuals

(Fig. 8) reveals numerous banding differences in these regions. In region *D 1 a–D 5 a* (Fig. 8) no attempt is made to compare the banding pattern. Pairing between the homologues in this region is not observed. Besides these dissimilar regions there are present many small differences which are interpreted as quantitative and qualitative in nature. The quantitative differences are of two types: (1) those in which a single or double band present in one homologue has no mate at the corresponding locus in the other homologue, and (2) those in which one member of a pair of bands is missing. A difference of the former type is the fan-shaped *end 1* of ocellaris. This region has no counterpart in the reynoldsi homologue. Likewise the heavy capsule at *end 2* of the reynoldsi segment 1–2 has no mate in the ocellaris region. At the corresponding locus in ocellaris, *A 4 b c d*, there are present several bands, a single darkly stained broken band, a pair of lighter stained thin bands and a single dark band. At *A 3 h* (Figs. 8 and 17) and at *C 5 c* two extra bands are present in the reynoldsi chromosome. The capsule-like structure at *B 4 a* (Figs. 11 and 12) in ocellaris is not observed in the other homologue. The other type of quantitative change frequently encountered in these preparations is that in which one member of a pair of bands is missing. At region *C 1 b* in the ocellaris chromosome two thin bands are present; in the reynoldsi homologue at this locus, a single band is seen (Figs. 8 and 17). In reynoldsi at *A 4 f* a pair of thin broken bands is observed and at the corresponding region in ocellaris a single solid band (Fig. 8). *B 3 f* in reynoldsi contains two bands, one dark and heavy, the other light and thin; in ocellaris a single dark irregular band is present (Figs. 11 and 12). In ocellaris, *D 6 g* contains a pair of heavily stained bands: one of these is broken and at this locus in reynoldsi a darkly stained structure which involves three thin bands is present (Fig. 8). Region *C 4 d* consists of a pair of bands in reynoldsi and a single band in ocellaris (Figs. 11 and 12).

Besides the small differences just described several cases have been observed which may involve structural (qualitative) modifications (not additions) of homologous bands. The evidence on this point is not yet clear. Examples of these possible qualitative differences are shown at *D 8 c* (Fig. 22), *C 3 d* (Figs. 12 and 14), *D 5 f* and *D 5 a* (Fig. 22).

Discussion

As already pointed out, previous investigations of the present species hybrids in Sciara revealed no gross chromosome rearrangements, particularly of the typical inversion type so common in Drosophila. The present investigation, however, shows that, although they are not numerous, rearrangements are found in chromosome *C* of this hybrid and also within *Sciara ocellaris* itself. This evidence indicates that there is nothing peculiar about the chromosomes or mitotic mechanism in the present material to prevent the spontaneous origin or perpetuation of rearrangements. However, the low frequency of gross chromosome rearrangements in the species of Sciara considered here is surprising in view of the relatively high frequency of such rearrangements in Drosophila and Chironomus and also in some other species of Sciara. In extensive studies of species hybrids and also of various geographical populations within individual species of Drosophila, inversions are the most numerous of the chromosome rearrangements observed (Dobzhansky and Queal, 1938; Dobzhansky and Sturtevant, 1938; Horton, 1939; Hughes, 1939; Patterson and Crow, 1940; Sturtevant and Dobzhansky, 1936). Similar conditions appear to exist in Chironomus (Bauer, 1936). In Sciara the recent studies of Carson (1944) and McCarthy (1945) show that in certain species inversions are found frequently in wild populations. The reason for the relative infrequency of rearrangements in the present material is not known.

Another item of interest is the relation between the broken reynoldsi chromosome *C* and the inversion of one

segment (1–2) observed in the hybrid. If the interpretation is correct that part of the difference between the ocellaris and reynoldsi chromosome *C* is due to an inversion of one of the segments of the reynoldsi chromosome, then the question as to whether or not the presence of the inversion is connected with the fact that the reynoldsi homologue regularly separates into two segments arises. At the present time this question can not be answered. Apparently the point of separation is near the spindle fiber attachment (centromere), but other V-shaped chromosomes in Sciara do not ordinarily exhibit such behavior (See Metz, 1939, for one exception to this rule).

In addition to the gross rearrangements described, there are many small differences. These small differences are commonly found in Sciara not only in natural populations of some species, but also in the ocellaris-reynoldsi hybrids where they are the only modifications thus far identified in the *A*, *B* and *X* chromosomes. In Drosophila, on the other hand, small differences are rare. A few have been noted in naturally occurring races of certain species and several have been described in connection with hybrid crosses (Dobzhansky and Tan, 1936; Horton, 1939; Hughes, 1939), but in contrast to those in Sciara they nearly all appear to be inversions. The significance of the small differences is as yet unknown.

No small differences have been detected in chromosome *C* or in any of the other salivary gland chromosomes in natural populations of *Sciara reynoldsi*. The lack of the small differences may be due to the small amount of material available, but the presence of the rearrangements in the reynoldsi chromosome *C* suggests that as regards chromosome rearrangements, *Sciara reynoldsi* may prove to be similar to Drosophila.

From this study it is evident that the same types of chromosome alterations occur in Drosophila and Sciara. But the relative frequency of the types of changes seem to be distinctly different in the two genera, at least as regards the minute modifications determined from *Sciara*

ocellaris and the species hybrids considered in the present paper.

Summary

1. An analysis of salivary gland chromosome *C* in *Sciara ocellaris* and *Sciara reynoldsi* is presented.

2. A description of a spontaneous inversion occurring in chromosome *C* in one stock of *S. ocellaris* is given.

3. Intraspecific matings of the three stocks of *S. ocellaris* show the presence of one small difference, a deficiency of two bands near *end 2* of chromosome *C* in the "Hammond" stock.

4. In the intraspecific matings of four stocks of *S. reynoldsi*, no small differences in chromosome *C* are detectable.

5. In interspecific matings between *S. ocellaris* and *S. reynoldsi*, chromosome *C* of reynoldsi differs from ocellaris by: (a) two independent inversions or one small transposition; (b) a short unpaired region; (c) small differences (deficiencies or duplications).

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ON THE SYNTHESIS OF GENE-PRODUCTS IN EQUIMOLECULAR QUANTITIES[1]

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THE extensive experiments printed as preliminary communications in 1939, 1940, 1941, are based mainly on investigations of chemical substances known in biochemistry after the works of Duclaux (1879), Hata (1909), Jacoby (1920, 1926), Euler and Svanberg (1920, 1921), Olson (1921), Myrbäck (1926) and others, as enzyme inhibitors.

It is very important that the poisoning of enzymes in biochemical experiments proceeds as a stoichiometric process (Myrbäck).

Among the morphogenic substances, there are many which are not enzymes. However, according to the hypothesis lying at the foundation of this investigation, the majority of gene-products are of an enzymatic nature. The experimental material under consideration proves this hypothesis advanced in genetics by Hagedoorn (1911), Morgan (1916), Wright (1916) and Goldschmidt (1916). More than half of the specific morphoses found under the action of chemical substances in *Drosophila melanogaster* and other species of this genus, have been caused by enzyme inactivators.

For instance, mercury causes the phenocopy of the Minute-type, repeating all the finest morphologic peculiarities of this mutation. Silver calls forth the phenocopy of the types yellow or straw; antimony—the phenocopies of the bordeaux or brown types and some other manifestations; boron—the phenocopies with some peculiarities of eyeless. Fluorine causes the occurrence of small melanistic inclusions under the chitin of the larvae and imago; nitrous acid—the phenocopy of the thick and thickoid leg types; sodium perchlorate—sterility, caused by the ab-

[1] A chapter from the dissertation "Phenogenetic analysis of differentiation" presented February 1941 at the Moscow University. During the war the author was in the Army.

sence of the external genitalia in both sexes; thiocyanate compounds—shortening of the body of the type chubby mutation; trichlorlactic acid—the phenocopy chrome; neutral red—intensive abdomen abnormal; p-aminobenzoic acid affects the structure of eyes, wings and legs. About thirty cases were found of the morphogenetic activity of enzyme inhibitors and a number of morphoses occurring under the influence of other substances as well. Very interesting among the latter is the action of tryptophane which not only acts on vermilion and cinnabar, as other authors have found, but in our experiments transformed the wild-type color of the eyes into an intense dark one. Only in the case of morphoses induced by the compounds of sodium perchlorate were the phenocopies found in 25 per cent of the individuals; in all the other experiments, the morphoses appeared in 100 per cent of the individuals developing in a medium containing definite concentrations of chemical compounds. All the modifications found were so highly specific that it was possible to investigate with their help also the quantitative relations regulating the synthesis of morphogenetic products during the embryonic period. It became possible to compare different morphogenetic products in vivo, when the pH of the external medium, the pH of body fluids, and other conditions are identical or do not vary to a great degree. These comparisons throw some light on the general peculiarities of the synthesis of morphogenetic substances in the organism.

Material and Method

As test object served the Naltchik stock of *D. melanogaster*. The comparative study of morphoses was made on twenty other species of the genus Drosophila. Larvae of the first and second instars of exactly determined age were placed into small vials containing a definite quantity of the tested chemical compound. However, the sensitive periods of almost all the changes set forth below occupy more than one instar. The food medium consisted of molasses, maize flour, etc., standard for our laboratory

and already described. Other recipes give slightly different results. The cultures developed at 25–27° C.

Experiments

The great number of cations and anions giving a clear effect make it possible to expect definite quantitative dependencies when active concentrations of different ions are compared. Only the data about well soluble compounds will be used for this purpose because our interest is not in the quantity of the chemical introduced in the medium, but only in the quantity which is dissolved. Universally acting concentrations should not be expected since different chemical inactivators form different compounds with morphogenic products. For instance, some combine with carboxyl, some with carbonyl, others with amino-groups of the morphogenic products, etc.

A considerable, prevailing number of modifications caused by inorganic stuffs permits one to postulate that the morphogenic enzyme products have a protein nature even before a direct analysis of these substances. The poisoning effectiveness both among the anions and among the cations shows that the inhibited enzyme compounds have an amphoteric nature characteristic for proteins, and salt-like linkages are formed with acid or alkaline groups.

Some substances, nevertheless, apparently react with chemical groups having the same structure in different morphogenic products and they are, therefore, in the possibly nearest concentrations. A comparison is given of several anions, cations and of two reagents for the carbonyl group.

It is of interest to compare anions of sodium borate, of thiocyanate and of fluorine. Sodium borate acts in concentrations of 1 : 750, potassium thiocyanate—in concentrations of 1 : 750–1 : 1500 and sodium fluoride—1 : 3000.

The comparison of modifications caused by perchloric acid[2] and characterized by incomplete manifestation, shows that the lowest acting molar concentrations of sodium perchlorate almost coincide with active concentra-

[2] The same modification is caused also by sodium hypochlorite.

tions of sodium nitrite. The perchlorate has a higher molecular weight than the nitrite.

For cations, a comparison of mercury and silver is given, owing to the qualitative inactivation peculiarities of these ions. If the larvae are kept for twenty-four hours in an active concentration of one of these ions and are subsequently placed into normal media, modifications will, nevertheless, take place. Thus, in both cases partly insoluble complexes of cations and of corresponding formative products arise. It should be remembered that enzymes also regenerate feebly from salt-like compounds with silver.

Mercury chloride causes morphoses in concentrations of 1 : 2000–1 : 3000, and silver nitrate in concentrations of 1 : 800–1 : 1250.

Two standard chemical reagents for the aldehyde group : hydrazine hydrochloride and semicarbazide hydrochloride act in exactly coinciding concentrations of 1 : 1000. Both these substances cause a pronounced melanic color, but semicarbazide causes also another morphological change.

It is astonishing that molar concentrations of ions affecting various morphogenic reactions either coincide or differ only by several times. The difference by two or three times may depend in some cases on the influence of other substances in the cells of the developing organism, an influence which cannot be excluded even when the principal reactions of inactivation are highly specific.

However, in many other fine physiological reactions, the range of the differences in similar comparisons is hundred and thousand times wider.

The most interesting and significant data may be produced in the investigation of highly soluble compounds when both anion and cation exhibit a pronounced morphogenic effect. An equal amount of dissociated active anions and cations appears in any concentration of these compounds.

For instance, the easily dissociating silver fluoride. Under the influence of sublethal concentrations of AgF

in vials the walls of which were covered with paraffin, and in ordinary vials, 109 imago were obtained having yellow chitin and yellow bristles characteristic for silver, and small dark inclusions reminding of the mutation benign tumor characteristic for the fluorine compounds. Such changes did not appear in the controls.

It should be mentioned that silver chloride, nitrate, sulfite, acetate, citrate, nucleinate, etc., give only the yellow-straw effect. The sodium, potassium, barium, cerium, fluorides, fluoric acid, fluoroiodine and other compounds of fluorine, cause morphologically only melanistic inclusions in larvae and imagines.

Of interest are the results of the action of partly soluble compounds of cations and anions as, for instance, mercury thiocyanate, only slightly soluble in food media. In other compounds the thiocyanate ion apparently also forms a partly insoluble complex with the morphogenic product, since larvae, kept in a medium with ammonium thiocyanate for 24–26 hours, after being washed and placed in normal media manifest the modification at the pupal and imaginal phases of development.

Despite the low solubility of mercury thiocyanate, both modifications develop in sublethal concentrations, attributed to each ion separately. Thus, also the slightly dissociating cations and anions have closely coinciding thresholds of effective concentrations.

What are the quantitative relations between concentrations causing a morphological effect and lethal concentrations?

A comparatively insignificant increase of a concentration causing a phenocopy calls forth a lethal result. Sodium borate is toxic in solutions of 1 : 600, semicarbazide —1 : 700, potassium thiocyanate—1 : 600. Similar data have been obtained for silver and other investigated compounds. Concentrations, causing phenocopies, have already been given. Concentrations causing a clear visible effect are, therefore, followed by concentrations with a lethal action.

Discussion

The influence of similar or equimolar concentrations of substances causing different phenocopies and, therefore, affecting various morphogenic products, may be interpreted as the consequence of synthetic processes, common for the cell as a whole.

Among various quantitatively dependent and even synchronic processes in the cell, the multiplication of genes and chromosomes is the most important or at least the most investigated. Characteristic for them are simultaneous and multiple augmentation and diminution of large complexes. The conclusion may be, therefore, drawn that the investigated enzyme-like morphogenic substances appear during embryogenesis in simple molar quantities under the influence of chromosome structures.

In different tissues the chromosome mass of the intensely functioning cells augments. For instance, the nutritive ovarial cells of many animals. Even the extreme specialization of the biochemical function of the glandular cells is, nevertheless, correlatively connected with an increase of chromosome threads in every cell, either the chromosome number increasing or the manifold reduplicated elongated threads transforming into a giant chromosome. We suppose, therefore, that enzymatic products synthesized under the influence of the genes, possibly enter the cells in equal molar concentrations.

About the relative vicinity of the concentrations causing visible changes in morphogenesis and of the lethal concentrations, the following should be mentioned.

The allelomorph series of many or even of the majority of gene loci contain both lethals and visibles. Some of these lethals are caused by deficiencies, but some give reverse mutations and are, therefore, caused by chemical rearrangements. Lethals prove the primary role played by genes in normal development and the lethal effects of separate loci are very specific. It would be fallacious to identify the series of multiple allelomorphs with various quantitative gradations of phenocopies, and a comparison

is merely given between a visible and lethal change. Each step of the multiple allele is a quantitatively singular chemical structure, and the gradation of phenocopies in the experiments under consideration is only the result of the inactivation of the unequal quantity of molecules in the morphogenetic substance of the wild type allele.

The proximity of the compared concentrations shows that the lethal action of the compounds used in the experiments is the result of the possibly strongest influence on this embryonic physiological reaction, whose lesser shifts are followed by phenocopies. A specific lethal action is present, which repeats the specific lethal action of the genes.

The similarity of morphologically significant and of lethal dosages of each substance proves that here the lethality is caused by more various toxic mechanisms than those characteristic for the poisoning of adult organisms.

Summary

Many specific phenocopies, corresponding to almost all principal mutation types, have been obtained by means of chemical enzyme inhibitors. Among the substances causing specific morphoses are the ions of heavy metals, inorganic and halogen substituted organic acids, hydrazine, aromatic ketons, ketenes, etc. Both the process of inactivation of morphogenic substances and that of enzyme inactivation depends on the quantity of enzyme inhibitors. The comparison of concentrations of several heavy metals, anions, organic enzyme poisons, shows that their effective dosages are either identical in molar concentration or closely coincide. The simultaneous occurrence of two morphoses under the influence of one easily dissociating compound, the first modification being caused by an anion and the second by a cation, may be regarded as a sufficient proof that the corresponding inactivated morphogenic products also form in the cell in equimolecular quantities.

Concentrations causing visible effects and those causing lethal ones appear to be adjacent. Thus, a lethal effect

may in this case be regarded as the result of the most strong and noxious quantitative change of the same reactions whose feebler shifts produce morphoses.

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BREEDING, REARING AND CARE OF THE SOUTH AFRICAN CLAWED FROG (*XENOPUS LAEVIS*)

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SINCE the adoption of the South African clawed frog (*Xenopus laevis*) as a routine laboratory test animal for the diagnosis of pregnancy, a demand for large numbers of them has arisen. To date, the only source of these amphibia has been their native habitat—South Africa. An adequate, more convenient source of supply of these frogs is needed. To this end, numerous attempts have been made to breed and rear these frogs in captivity, thus providing a source of supply of them as readily available as for other types of laboratory animals.

The first clear-cut progress toward this goal was achieved in 1936 when Shapiro (1936) described the production of amplexus (copulatory embrace) and fertile eggs by the administration of gonadotropic hormones to male and female frogs. With this technic it became possible to obtain tadpoles at any time of the year without reference to the animals' mating season or environment, since the female frog contains mature ova at all times, extruding them only upon amplexus with the male or upon artificial stimulation by gonadotropic hormones. Using these procedures Gasche (1943) and Aronson (1944) have reported the successful breeding of *Xenopus laevis*. To the best of our knowledge, however, no report has been made yet of the rearing of these frogs to full adult size, and considerable doubt has also been raised as to whether

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frogs bred and reared in captivity will be fertile or adequate for pregnancy diagnostic work (Aronson).

During the past two years' experience with the South African clawed frog at the Mallory Institute of Pathology of the Boston City Hospital, two captive generations have been bred and successfully raised to full maturity. In the course of this work many technics have been evolved which greatly simplify the rearing of these amphibia, to the point where the procedure now appears to be sufficiently simple so that any well-equipped routine laboratory might undertake it with reasonable assurance of success. This material will be discussed under the following headings:

(I) Injection and mating of frogs.
(II) Rearing of tadpoles to metamorphosis.
(III) Care of maturing frogs.
(IV) Care of adult frogs.

I. Injection and Mating of Frogs

In their native habitat these amphibia have definite mating seasons when the male frog enters into amplexus with the female, stimulating thereby the extrusion of eggs which are fertilized by the male as the eggs pass his cloacal orifice. As Shapiro (1936) demonstrated, the production of this amplexus in captivity can be accomplished at will by the administration of gonadotropic hormones. These hormones may either be of chorionic origin obtained from placental extracts or concentrates of pregnant urine, or they may be of pituitary origin obtained from extracts of animal pituitary glands. In this laboratory we have found gonadophysin, a stable commercial extract of sheep anterior pituitary (prepared by G. D. Searle and Company, Chicago),[4] to be the most satisfactory source of this hormone.

One cc of a saline solution of this powdered extract containing 200 rat units is injected into the dorsal lymph sac of the male and female frog. The size of this dose is

[4] We wish to acknowledge our indebtedness to G. D. Searle and Company who kindly supplied the gonadophysin used in our experiments.

considerably in excess of the minimum amounts required to evoke a reaction in the male and female, but it has been arbitarily set at this high level to avoid the complication of non-reaction in a rare physiologically sluggish frog. This relatively large dose of hormone did not appear to be the cause of premature oviposition, since in several instances eggs did not appear until 48–72 hours after administration of the hormone. In a series of matings various time intervals between injecting the male and female animals have been tried, at times injecting the male 1–2 hours before the female and at other times the female 2–6 hours prior to the male. Viewed in retrospect, such spaced injections proved to be no more fruitful than the simultaneous injections.

Following the injections the mated frogs were placed at once into a previously prepared aquarium. The following factors appear to be important in the preparation of this aquarium:

(1) Only tap water stored for at least 48 hours should be used. This storage time permits the volatilization of any contained chlorine, a substance lethal to tadpoles even in minute dilutions.

(2) Thermostatic control of the temperature does not appear necessary since ordinary room temperature, 20–28° C., appears to be adequate, and the tadpoles are remarkably resistant to even fairly marked changes in temperature.

(3) The aquarium should not be filled to a depth greater than 8–9 inches, providing thereby at least some surface oxygenation of even the deepest layers of water.

(4) The surface of the water in the tank should be at least partially covered with some type of floating plant life having submerged rootlets. *Salvinia natans*, a common aquatic plant readily available in most tropical fish stores, was found entirely satisfactory. The roots of these plants provide sites of attachment for the fertilized ova immediately below the surface of the water where high oxygen tensions exist.

(5) If convenient, a slow bubble of oxygen or air through the water may contribute to the percentage yield of developing eggs; it does not appear, however, to be essential to the outcome (4).

It is well to maintain a quiet, darkened environment for the mated animals in the aquarium, since external stimuli appear to startle them, occasionally causing premature interruption of amplexus. Under usual circumstances, if the frogs are injected in the late afternoon, amplexus may be observed in the early evening (Fig. 1). At this time they may be seen swimming about the tank in and about the plants, in constant amplexus, infrequently with their

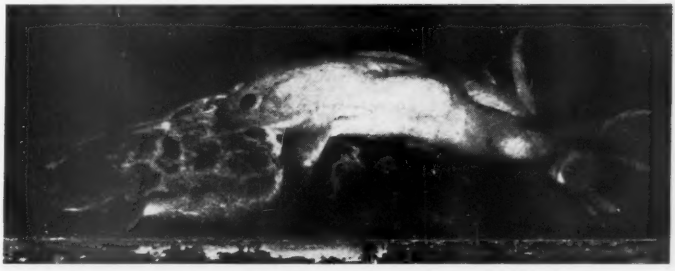

FIG. 1.. Adult frogs in amplexus 18 hours after administration of gonadotropic hormone.

ventral surfaces uppermost. Eggs, frequently appearing 8–12 hours after injection, are thus deposited on the rootlets to which the mucoid capsules adhere (Fig. 2). The number of fertile ova obtained in any mating is extremely inconstant, varying from less than a hundred up to possibly thousands. Many of the fertilized ova do not become attached to the plants and fall to the bottom, and only rarely will these non-attached eggs develop. Despite the early appearance of eggs in the tank, it is advisable not to disturb or remove the mated frogs for at least 24 hours because ova may be extruded and fertilized throughout this period of time, notwithstanding apparent intermittent interruptions of amplexus. Occasionally eggs may not appear within the first 24 hours, appearing dur-

ing the second day. Therefore, if the mating produces no ova within the first 24–48 hours, the frogs should be permitted to remain in the mating aquarium for 3–4 days, on the chance of encountering a delayed response.

It must be emphasized at this point that not all matings produce fertile ova. In our experience, the incidence of successful matings was approximately 1 in 4. The reasons for these numerous failures are completely obscure. Rarely the female frog failed to react and no eggs were obtained. At other times amplexus failed to develop and

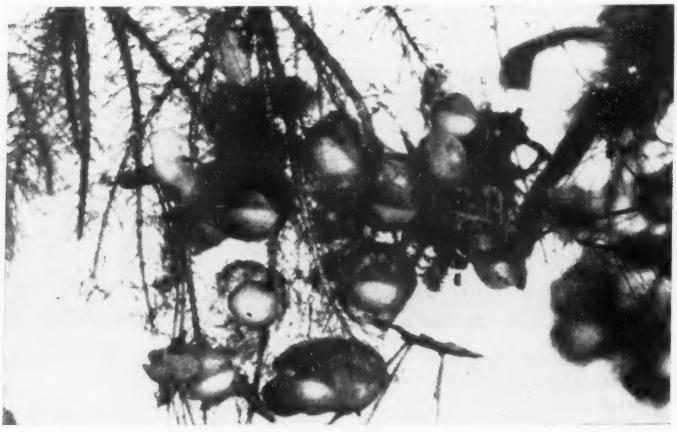

FIG. 2. Ova 24 hours after oviposition attached to rootlets of floating plants.

abundant ova were found but none were apparently fertile. These failures could not be correlated with the season of the year, size of the frog, temperature or pH of water, or interval of time between injections of the male and female animals.

REARING OF TADPOLES TO METAMORPHOSIS

Once the adult frogs have been removed from the aquarium containing the fertilized eggs, nothing further need be done to the aquarium for 2–3 days, save possibly for the continuance of the oxygenation. Within 36 hours

a definite increase in the size of the ova can be noted, and in 48 hours leaf-shaped embryos can be seen within the egg membrane. One day later free swimming forms may be found (Fig. 3). Once the larvae become free-swimming, it is advisable to remove the floating plants because the albuminous mucus extruded with the eggs fouls the water.

During the succeeding 3–4 days no food should be added to the water because at this stage of development the larval forms, while still absorbing their yolk sacs, feed on dissolved substances from the infertile eggs and minute plant life contained in the water. Beginning approximately one week after the mating, the developing tadpoles require periodic feeding. There is considerable difference of opinion among the various investigators concerning

FIG. 3. Tadpole 3 days after hatching. (×5)

the food habits of the tadpole. The chief point of controversy centers about whether the tadpole is essentially a herbivorous or carnivorous animal. In these breeding experiments we have fed the developing forms, at one time or another, dried, powdered clover meal according to Gasche's technic (1943), infusorial cultures of Chlamydomas, Eugenia and Paramecia according to Bles' description (1901), and finally settled upon finely powdered dried beef and lamb liver. Serial microscopic sections of the tadpoles were performed during these feeding experiments and the intestinal contents of the tadpoles noted. Without any doubt, when the water contained infusoria and ground clover meal both substances were found in the intestinal tracts of these animals, notably indicating that their food habits are far less exacting than was formerly believed, and suggesting that the tadpole strains and ingests from the water any organic material found therein.

The dried powdered liver was found to be both a simple type of food to use as well as an excellent feeding substance. It was added to the aquarium by merely sprinkling the powder on the surface of the water where it gradually absorbed water and sank to the bottom. The tadpoles could be raised on this substance solely, from the time of their first feeding to their complete metamorphosis. It is probable that the powdered liver, besides serving as a food substance in itself, also provided an infusorial culture medium upon which organismal life thrived, providing thereby further food for the tadpoles. The amount of liver required and the frequency of the feedings were very relative matters depending entirely upon the number of tadpoles in the tank. The best criterion in this connection was the clarity of the water. As long as the water was crystal clear, albeit somewhat discolored by dissolved substances, additional food could be added. It was found best to start off with small amounts of the liver powder (a half gram or less per aquarium) every 3–4 days and increase the volume and frequency of the feeding as rapidly as the clarity of the water permitted. The general principle that underfeeding was far more to be preferred than overfeeding was adhered to throughout their development. No ill effects have been noted from week-long intervals without feeding. At the same time, persistent underfeeding should be avoided since the rate of development of these young tadpoles may be seriously retarded. By the use of the above described feeding methods, the water required no periodic changing or filtration, and the bottom debris which collected in very small amount required no removal, since the animals themselves were effective scavengers, keeping the aquarium adequately clean. On occasion, mold growth developed on the walls of the tank as well as the surface of the water; these were clear signs of excess feeding. This growth may become so heavy as to require mechanical removal and should suggest the need of decreasing the volume of future feedings. Another sign of excess feeding

was given by the tadpoles themselves. When the water became fouled with food, they became sluggish and floated on the surface. At times like these, oxygenation of the water was apparently beneficial, but otherwise no benefit was noted from oxygenation of the water after the first week of life of the tadpole. Nor was the addition of algal growth to the water any help. Sand which was layered over the bottom of the tanks in early matings was soon discarded from the aquarium in later matings as contributing no desirable effect. In brief, the only care which the aquarium required was the periodic addition of stored water to make up for evaporation losses, and the only care the tadpoles required was a periodic addition of powdered

FIG. 4. Tadpole showing hind limb buds as evidence of beginning metamorphosis 5 weeks after hatching.

liver. Under this simple regime metamorphosis was noted within 5 weeks and was complete within 9–10 weeks (Fig. 4).

The problem of how many tadpoles should be kept in any given aquarium was a difficult one. No definite relation between numbers of tadpoles and volume of water was established. In general, however, not more than 200–250 tadpoles were raised in a single 25-gallon aquarium. As has been demonstrated repeatedly by others, overcrowding significantly retards their rate of growth and metamorphosis.

CARE OF MATURING FROGS

The period during which the tadpoles are metamorphosing is one of the most critical in their lives, so it is

important to look out for, and promptly remove to another aquarium, any young frogs seen squatting on the bottom. No longer will infusoria or powdered liver suffice. They now require either strips of beef heart or white worms (*Enchytraeus albidus*) of which they will consume astonishing quantities.[5] From the time that they first begin to resorb their tails, the little frogs are capable of mastering earthworms and finely shredded liver or beef heart, often of such considerable volume as to create obvious external abdominal distention.

Previous writers have suggested feeding Xenopus on finely minced meat, but this disintegrates so rapidly in water that much is lost and the water soon fouled. More satisfactory results can be obtained and the water not fouled by cutting the meat, while still frozen, into worm-like strips. This cutting is a time-consuming business where many frogs have to be fed, and in such circumstances a slicing machine of the kind used for cutting bread or rashers of bacon is best. After slicing the frozen liver or heart to the desired thickness, the slices should be restacked and put through the machine again though on a different plane so as to produce worm-like strips.

[5] Fortunately these worms breed rapidly if provided with congenial conditions. To obtain the best results one or more china or glass receptacles are necessary; small, cracked aquaria will serve the purpose. The vessel should be filled to a depth of 2 or 3 inches with moist earth. On the earth should be spread a cupful of cold, cooked oatmeal porridge. Then introduce the culture of worms and they will feed around the edges of the porridge and burrow between it and the earth. The important thing is to keep the moisture content of the earth just right; if too dry the worms will desiccate, and if too sodden they will drown or ascend the sides of the vessel. It may be found convenient to keep a bottle of water, fitted with sprinkler stopper, handy to the terrarium. Desiccation can be retarded by *almost* covering the terrarium with a piece of glass. If entirely covered, humidity will foster mold (a moderate amount of which does no harm) that quickly smothers everything. It does not seem to matter if the porridge turns sour but it will require replenishing from time to time, depending on the number of worms consuming it.

To find the worms, raise or roll back the porridge and scoop up the worms with a spoon. To separate them from the earth and drop them into the aquarium, a pair of fine-pointed forceps will be found indispensable. During the summer months some gauze or screening should be slipped beneath the glass cover so as to exclude the tiny fruit and other flies which, attracted by the sour porridge, soon foul the terrarium with their maggots.

To supply the calcium necessary for bone building, a lump of plaster of paris should be kept in every aquarium where it will dissolve in the course of a month or two and require replacing.

It has been said that the aquaria should be subject to bright sunshine for a short period each day. Any sunshine that may have reached our frogs has done so through double glass, *i.e.*, of the window and aquarium. So persistently did our Xenopus seek out the darkest part of their aquaria when exposed to ordinary daylight that for almost two years we have kept the white cotton shades permanently down in that half of the room where the aquaria are near the windows. The aversion to light displayed by these captive frogs is doubtless in part due to the absence of aquatic plants or other cover in their aquaria.

In agreement with Bellerby (1938), our frogs appear none the worse for being kept in a dull light, and in this connection it should be remembered that one form (*Xenopus laevis borealis*) has been found living in dark caves on Mount Elgon, Kenya Colony, where allegedly they had no light whatever. On the other hand, in parts of Tanganyika Territory where the nights are cold but the days hot, many Xenopus have been seen resting on the surface of the water in the full glare of the forenoon sun, apparently for the sake of warmth. In captivity they thrive at temperatures of about 70° F. ± but stand fluctuations well. They need to be guarded from the extremely high temperatures of midsummer in the United States.

During the first few months of their life some frogs grow so much more rapidly than their fellows that the resulting disparity in size is often surprising. Even though the frogs are being fed daily, it is advisable, in order to frustrate attacks on the smaller ones by their cannibalistically inclined companions, to segregate them into two or three aquaria according to size.

Relative to the need for periodically changing the water in the aquaria, some difference of opinion exists. Aronson

believes that 3–4 changes a year are sufficient. In our experience the change 3 times a week has been performed with little difficulty and the animals have thrived so well that we have continued this practice.

While the frogs are very small it will be found necessary to screen off a corner of their aquarium with fine mesh netting to prevent the little amphibians being sucked up by the rubber tube used to siphon off the dirty water. To run in the fresh water straight from faucet to aquarium would be to subject the frogs to unnecessary cold shock. Our custom, therefore, has been to draw the water off and allow it to stand for two days to permit of its assuming room temperature as well as to undergo dechlorination. For the two-day storage period we have found a couple of 50-gallon wood hogsheads eminently satisfactory. Installed on boxes that raise them above aquarium level, they are filled by a rubber hose connected to the faucet.

So long as it is over 6 inches deep, the precise amount of water in an aquarium is immaterial for Xenopus thrive in Africa in pools of varying depth, passing much of their time buried in the mud at the bottom. They will leap out of an aquarium if the water is too shallow. One night a male sprang from an aquarium $14'' \times 8'' \times 10''$ high as there was only 4 inches of water in the bottom. They become restless and anxious when the water is low. Similarly, there is danger in having an aquarium too full unless it is covered.

Care of Adult Frogs

Much that has been said regarding the care of developing frogs applies equally to the adults. The daily feeding of the very young gives place to feeding the half-grown on alternate days, but the adults only twice a week except at certain seasons when the frogs indicate that they are hungry. They do this by swimming to the glass side of the aquarium nearest to any one who may approach. Then, with their slender tactile fingers, they make rapid motions as if guiding imaginary food into their mouths. Females

exhibit a better appetite than males; this is particularly noticeable after they have spawned. So accustomed do they become to being fed that they vie with each other in seizing meat strips from one's fingers, and even leap out of the water in their eagerness or as an expression of vitality. A few individuals, even though full-fed, will swim to and rest contentedly upon a hand partly submerged at the surface. The nutrition of these adults must be carefully and adequately maintained, since without doubt malnourished animals may become the annoying source of falsely negative tests.

When they become tame it greatly facilitates their removal from the aquarium, some even swimming into the reddish net used to transfer them. This net, especially made for the purpose of sweeping along the floor of the aquarium, consists of a wide-mesh onion-bag mounted on a stout wire frame. The chief peculiarity of this frame is its one straight side rising in a semi-circle to the short wooden handle. The obvious advantage of the straight side is that it can be applied closely to the aquarium's floor, consequently making it more difficult for the frogs to elude. The depth of the bag is 10 inches, just deep enough to enable it to be closed over on the frog by a turn of the wrist.

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THE INDUCTION OF GERMINAL MUTATIONS BY CHEMICAL MEANS[1]

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THE production of germinal mutations affecting somatic manifestations such as coat color, eye color and distributional patterns of pigmentation was the result of the practical application of genetic principles by which the attempt was made to duplicate in mice, under controlled conditions, the obviously biologic variable state of the human population. In order to do this, the processes of hybridization and the elimination, by selection, of unfavorable sublines that did not conform to type, were used. These same genetic procedures alone, which have been employed by the present author for more than 27 years on a population of more that 210,000 mice, had produced very few spontaneous germinal mutations—8 in all, or a mutation rate of 1:26,250 mice. Consequently in order to increase germinal mutations some other modification of the above program was indicated. Since methylcholanthrene is recognized by all investigators as a very powerful carcinogen and since the process of the origin of cancer may be in the nature of a stimulation or an alteration of biologic processes, yet unknown, within the cell, it occurred to me that this chemical may be a valuable tool for the investigation of general biologic problems in addition to the study of its ability to induce cancer. The results obtained in the production of many altered embryological, physiological and morphological characteristics have exceeded expectations. The exact details of the experimental procedure are as follows:

MATERIALS AND METHODS

Mice of three well-established inbred strains of the au-

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thor, the CBA, the N and the JK, were serially bred to each other and their lineal descents have been continued for 25 generations of a brother-to-sister mating following the last outcross. That is, a mouse of the CBA strain was outcrossed to a mouse of the N stock, thus giving rise to the CBAN stock. Before there could be much reduction of biologic variability introduced by the above process of hybridization, a mouse of the partially inbred CBAN strain was outcrossed to a mouse of the JK stock. This derived stock received the symbol NH. The progeny of this outcross were divided into four sublines, three of these were continued as controls in three separate laboratories, (1) that of Drs. Hooker and Pfeiffer, Yale University School of Medicine; (2) Dr. Kirschbaum, now of the University of Minnesota, and (3) in the laboratory of the present author. The fourth group of the NH strain were injected with methylcholanthrene beginning in the F_4 generation, that is, at the level of inbreeding to which the above three control groups had been subjected. Eventually the experimental group consisting of mice which had been injected with methylcholanthrene for many generations have been divided into many lines and sublines, some of which have been continued with the injection of methylcholanthrene into both parents in each and every succeeding generation while others have been continued free from any further injection of methylcholanthrene (untreated descendants of mice whose ancestry had been injected with methylcholanthrene for many generations).

In the experimental group, mice were injected subcutaneously in the right flank at 60 days of age with 1 mm of methylcholanthrene dissolved in 0.1 cc of sesame oil. All mice, both the experimental and the controls, have received the same standard diet of Nurishmix (Pratt Food Company) to which has been added a supplement of whole oats, wheat, sunflower seed and calf meal pellets. The mice have also received, twice a week, a slice of enriched Bond bread and fortified milk to which has been added Squibb's cod liver oil. Periodically the mice also received a supplement of lettuce, once a week, which had

been thoroughly washed with cold running water. All mice have been kept in the same air-conditioned laboratory in which the humidity as well as the temperature is uniform throughout the year (humidity between 50–60 per cent and the temperature 72–74° F.). Thus a strict adherence to the principles of quantitative research has been followed throughout the experiment, that is, that all variables, except one, were stabilized or standardized as near as possible. In this case, the only demonstrable variable was the expected biologic variability of the mice dependent to a great extent upon genetic variability brought about by hybridization and selection. All mice receiving methylcholanthrene were periodically examined for the appearance of tumors and the presence or absence of obvious disease as indicated by roughing of the hair, emaciation, soft feces around the anus and wheezing. All mice that showed a progressively growing tumor or the presence of disease were killed and all pathological lesions saved and fixed in Bouins fixative. These sections were stained by either hematoxylin and eosin or by one of the special stains. Mice that died during the extent of the experiment were also autopsied and the cause of death determined when possible. No normal mouse has ever been killed or discarded in either the control or the experimental series. Consequently some of these mice living more than a thousand days following the subcutaneous injection of methylcholanthrene and their descendants and those derived from relatively less resistant-to-tumors mice, whether untreated or injected with methylcholanthrene, have shown many phenomena of biologic significance including these germinal mutations that are being discussed in this paper. This long survival time of mice following the subcutaneous injection of "an overwhelming dose" of a very powerful carcinogen, methylcholanthrene, was not obtained at first in the early hybrid generations, but was the result of a selection-toward-resistance regime that was instigated from the F_8 generation onward. The selection-toward-tumor-resistance subline of the NH strain was now given the symbol NHO. The selec-

tion toward resistance was based upon (1) the continued breeding of the descendants of the most resistant-to-cancer pair of mice in each generation, (2) the continuation of the descendants of the subline whose ancestry, for several generations, had shown the greatest averaged latent period for induced tumors and (3) the application of the progeny test when the data on the descendants of any given pair were complete enough to warrant a genetic classification of the grandparents and great-grandparents.

When the germinal mutations appeared in these sublines where selection toward resistance to all types of tumors had been successful in increasing the average latent period for the appearance of tumors, they were treated as follows: (1) the new mutation was bred to a close relative and the mutant was established in the homozygous state; (2) it was outcrossed to an unrelated stock mouse to prove allelism or free assortment; (3) the F_1 generation mouse containing the new mutation was back-crossed to the recessive or the dominant condition as the case may be and a selection experiment toward the suppression of the increased incidence of the mutant continued for several generations to see whether a Mendelian 1:1 ratio could be disturbed, so that it could be indicated genetically if non-disjunction had taken place; (4) all new mutants were continued through their normal life span to see whether any somatic variation occurred with advancing age; and (5) when a recessive mutation occurred to back-cross it and its siblings to the parents in order to prove or disprove whether both of the parents were heterozygous for the new mutant. The later procedure was not always possible since by this time one or the other or both parents had died of an induced tumor.

Results

The regime of a genetic selection toward resistance to all induced tumors leads to a kaleidoscopic shift in the types of tumors. These induced tumors fall within four classes: (1) those appearing at the site of the injection

of the carcinogen; (2) those appearing on the surface of the body at sites removed from the site of injection; (3) those which appeared in the thoracic cavity; and (4) those which appeared in the abdomen. Beyond the stage in which late-appearing abdominal tumors appeared there was obtained a state, of short duration, in which no tumors appeared. This kaleidoscopic shift of tumors occurred in the succeeding generations (from the F_4 to the F_{22}), but there was a considerable overlap not only in generations of mice but also in the frequency distribution of the types of tumors. Following the attainment of the resistant state in which no tumors appeared in any part of the body of the mouse, after the subcutaneous injection of methylcholanthrene there was produced a reversal of susceptibility to induced tumors leading back through stages four, three, two, and finally one. This reversal took place in spite of the fact that a drastic regime of selection toward resistance to tumors continued to be the rule. In fact, during this "rebound" period tumors occurred even at the site of injection of the carcinogen earlier than they ever did in the original hybrid generations and some of them occurred so early that it was impossible to continue the stock by the fact that no progeny could be raised. The rebound period has occurred in all experimental lines except one. Here the selection toward a greater resistance to tumors is still positive; the latent period having been increased by selection from 126 days to 386 days over a period of 14 generations of a brother-to-sister mating. The rebound period has occurred at different levels in the different sublines. By this is meant that some of the sublines have never gone beyond stage 1 by genetic selection toward resistance, others have reached stage 3 and three of them have reached stage 4.

When the mice reached stage 4 in which abdominal tumors were the most frequent type, germinal mutations involving coat color, eye color, and distributional patterns of pigmentation became evident for the first time.

In two series of germinal mutations (of independent

origin) obtained in this experiment, there has been found a graded series of hair color ranging from ultimate dominant black through the well-established recessive brown to three levels of pigmentation below brown. These have been named descriptively (a) black, (b) pale black, (c) graphite, (d) dark gunmetal, (e) gunmetal, (f) darker bronze, (g) dark bronze, (h) bronze, (i) dark sepia, (j) sepia, (k) the original well-established brown, (l) chestnut, (m) light brown, (n) lighter brown, (o) velvet, and finally (p) buff. Each of these mutants breed true and they probably form a series of alleles at the brown-black locus. Many somatic mosaics have been obtained between two of the above mutant types, such as between chestnut and brown or brown and buff, etc. Due to the great number of mutants obtained within a relatively brief time, it is obvious that an unstable genetic state exists in these two lines one of which was an outcross between the methylcholanthrene injected series and C_{57} blacks, and the other, from a single litter of methylcholanthrene treated mice (without an outcross) in which three black mutants (graphite) occurred in a litter of seven young. Since a majority of the germinal mutations have occurred in these two descents (out of a total of eleven descents continued) the mutation rate from these two unstable genetic states is considerably higher than 1 in 557 estimated for the entire methylcholanthrene injected descent.

Many of these new mutations not only breed true, but they also retain a uniform pigmentation throughout life. However, some of them gradually shift toward the recessive condition (never toward the dominant) in the successive moults reminiscent of the shift of a black mouse, particularly if it be heterozygous for brown, shifting toward brown in old age. The offspring of these mice, which shift toward the recessive, start life at the level of hair pigmentation possessed by the parents at the first growth of hair. Thus there is indicated a genetic upset in the production of many new germinal mutations as well as

an upset in the somatic manifestation of pigmentation. Recessive brown (bb) has mutated to dominant black (graphite) eight times, three in the single litter referred to above and on five separate or independent times. In order to test out the possibility that this mutation rate may be high enough to distort a Mendelian ratio, mice of the experimental brown non-agouti subline were outcrossed to the normal black non-agouti C_{57} inbred stock. Of the 1,114 F_2 mice produced 833 were black, 1 bronze and 280 were brown. Thus the mutation rate to black (if any) was not sufficient to distort a 3 to 1 ratio (expected 3 : 1 = 835.5 Bl : 278.5 Br). However, two unexpected mutations were obtained, these were (1) bronze and (2) piebald (recessive). Neither one of these was in the ancestry of either parental stock and therefore must be considered mutations.

There has also been obtained a series of color mutations in which the hairs on the ventrum are colored differently than the hairs on the dorsum—this in spite of the fact that the ancestry for many generations had been brown non-agouti where there is no difference of pigmentation on the ventrum and dorsum. Thus a series of ventral hair colors have been established as follows: (a) pale black, (b) gunmetal, (c) rusty, (d) chestnut, (e) derris, (f) tan, (g) buff and (h) cream. There may be several other mutations which have not been classified as yet, such as a ventral chestnut streak between an otherwise buff belly.

Another series of mutations has given (a) white ears in contrast to ears of the same color as the rest of the mouse, (b) yellow scrotal hairs in contrast to the normal brown or black hairs, and (c) white areolar hairs, which have so far occurred only in females.

Another series of mutations has involved the production of spots. These are variations some of which have been reported in the literature but have never appeared in my own laboratory before. In addition to the white-haired blazes on the forehead and white spots on the

ventrum, the new mutants have given patches made up of the following hair colors: (a) yellow, (b) gray and (c) a ghost pattern. On the ventral side, left-handed spots, right-handed spots, mirror-imaged spots and dissimilar left-right handed spots have occurred and in these cases there is a delicate line of pigmentation in the middle of these ventrally occurring patches. All these types have been established in independent lines.

Another series of unexpected variations has also occurred in the descendants of mice whose ancestry has been injected with methylcholanthrene. These consist of the appearance of 15 recessive characters (13 pink eye and 2 piebald) where there had been neither of these well-known recessives in the ancestry for many generations. These have not been considered mutations unless evidence is forthcoming that at least one of the parents was not heterozygous for the particular recessive. This evidence is not always possible since one or both parents may have died of an induced tumor before their genetic constitution could be proven. Upon 11 occasions a pair of dark-eyed mice which had produced nothing but dark-eyed offspring (total of from 31 to 53 offspring) would produce a litter in which one pink-eyed offspring would be found. In two of these cases evidence that one of the parents was not heterozygous for pink eyed was produced and therefore these two cases of pink eye should be included among mutations.

General Discussion

The manner in which methylcholanthrene, and the other carcinogens, bring about the origin of a tumor or a cancer in an experimental animal is quite mysterious. It is still not known whether the inducing material is the originally injected methylcholanthrene or one of its metabolites. There is also the possibility that the injection of methylcholanthrene may release some biologic or chemical agent within the body which may be the true carcinogen. It is a generally accepted opinion that the action of the carcinogen is a local one upon the tissues which respond to neo-

plasia. A multiplicity of tumors have been produced, not only by injecting methylcholanthrene into different parts of the body and by changing the dosage or the solvent, but also, in the present investigation when the biologic variability has been considerably increased through the genetic processes of hybridization and selection. If one would only get histological modifications of cancer following the injection of methylcholanthrene, one biologic concept of the origin of cancer would be indicated. However, one also obtains many embryological alterations such as situs inversus, dextrocardia and identical twins (discussed elsewhere), many physiological alterations such as an increased or decreased growth rate of the animals and the production of giant and dwarf mice, alterations of fertility and fecundity, sexual maturity, senility, etc. (discussed elsewhere), and these germinal mutations influencing coat color, eye color and distributional patterns of pigmentation. Therefore, it is desirable to arrive at the conclusion that the administration of methylcholanthrene into experimental mice is serving as a valuable tool for the investigation of many problems of general biologic interest, other than that of the origin of cancer alone. Certainly the origin of cancer can not be dissociated completely from the other biologic phenomena being obtained. Until we know more of the metabolism of methylcholanthrene within the organism these problems can not be completely investigated.

Many of the present mutations have occurred spontaneously in other laboratories. They are, therefore, not unique, genetically speaking, but they have in the present experiment occurred at a far greater rate than can be expected by chance alone. Many of the mutations have occurred from an unstable genetic state. Unstable genetic states have occurred spontaneously and have been reported by several investigators. However, it is not likely, although not impossible that the two unstable genetic states reported here may have occurred entirely independent of the presence of methylcholanthrene or one of

its metabolites. Many of these mutations obtained from this unstable genetic state have also occurred independently in this series. Since the mutation rate here is approximately 1 to 557 with injection of methylcholanthrene compared to a spontaneous rate of 1 to 26,250 mice in the same laboratory and in the same genetically related mice, this increased mutation rate must be correlated with the presence of methylcholanthrene or one of its metabolites. Several germinal mutations changing susceptibility to fibrosarcoma induced by methylcholanthrene and the subsequent appearance of spontaneous adenocarcinoma of the stomach (discussed elsewhere) have also been obtained in this same series.

Germinal mutations have been obtained experimentally by x-rays, by ultraviolet light and, very recently, by the nitrogen and sulphur mustards. In the case of x-rays and ultraviolet light there seems to be an adequate physicochemical explanation of their action upon the germ plasm. X-rays, acting as a source of energy, break chromosomes, thus leading to new spacially related genic systems, to deletions, etc. Ultraviolet light, of a wave length of approximately 2600 Å units, is selectively absorbed by the nucleo-proteins of the chromosomes (genes). Many of the effects of the nitrogen and sulphur mustards on tissue culture and embryological development are similar to those produced by x-rays and thus it is not inconceivable to conclude that the effect of these chemicals upon the germ plasm is one of radiation. In the case of methylcholanthrene we do not now have an adequate physicochemical explanation. However, this may be forthcoming when the problem of these new induced germinal mutations is more fully investigated.

REVIEWS AND COMMENTS

EDITED BY CARL L. HUBBS

In these reviews and notices of current biological publications emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution. REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as indicated, all items are prepared by Dr. Carl L. Hubbs, Scripps Institution of Oceanography, University of California, La Jolla, California. All opinions are those of the reviewer.

Luther Burbank—A Victim of Hero Worship. By WALTER L. HOWARD. Waltham, Mass.: The Chronica Botanica Co.; New York: G. E. Stechert and Co. Chronica Botanica, 9 (5/6), 1946: 299–506. $3.75.

THIS BOOK, the product of a decade or more of labor, is a meritorious contribution to history. In the main the author has accomplished his avowed objective, *viz.*, to obtain the facts and to make a fair and impartial evaluation of Burbank and his accomplishments. That this was a worthy objective will be obvious to any one who reads this book with an open mind. Burbank's name became a household word and the Burbank myth, along with stories of Washington and Lincoln, is still perpetuated in many of the public schools of America, to tens of thousands of children, according to the author, with no end in sight. Therefore, even though the value of Burbank's work to science may not be impressive, it is fortunate that a true record of the life and accomplishments of this remarkable man has been made available. It should be required reading for every public school teacher and it is worthy of perusal by any biologist. The general plan of the book is stated as follows:

> In relating my human interest story of Burbank no attempt has been made to write a conventional biography. . . . My aim has been to explain and clarify some of the principal episodes of his life. . . . These encompass the main events of his life, most of them controversial. . . . For purposes of driving home important points, repetition seemed unavoidable. More than one episode might be employed to emphasize the same point: for example, the subject's egotism.

After an introduction and a chapter on background of the author, the episodes are presented in the following

sequence: Burbank, the man, the nurseryman, the scientist, the egoist, the mentor of youth, the unfortunate, the pariah—of scientists, the disappointed, the world character, the individualist; then follow chapters on Burbank's ethics, his religion, his foray into science—the Carnegie grant, his admirers, his detractors, and his place in the hall of fame. The volume includes a 33-page summary of Burbank's productions, and two short chapters entitled Aftermath and The Burbank Family. The short bibliography is supplemented by copious footnote citations to literature. There is a good index.

For biologists, the paramount question concerning Burbank is his relation to science. The author's discussion of this question is, for the most part, an unbiased factual presentation. To the present reviewer Burbank once stated that he had gotten all his inspiration by reading Darwin. This agrees with his purported statement that it was *Cross- and Self-Fertilization in the Vegetable Kingdom* that opened his eyes to the possibilities in plant hybridization. "One sentence in the very introductory chapter of that volume opened the door of my mind and took possession of my fancy . . . Darwin was writing of the plants themselves—I was thinking of mankind. If Nature had developed an incredible system by which plants could re-create and diversify and improve themselves *for their own benefit and advantage*, why should not Nature be induced to employ the same system *for the benefit and advantage of man?* It was my starting point." Thus plant improvement became the great ruling passion of his life and it cannot be said too often that he believed in himself and his mission. Of course there were other stimuli. But primarily it was Burbank's curiosity which spurred him on until he had experimented with thousands of species in nearly 200 plant genera, 121 of which yielded new forms deemed worthy of introduction as horticultural varieties. In spite of all the duds, the disappointments experienced by purchasers of many of his introductions, Burbank's contributions to horticulture are of outstanding impor-

tance. Of fruits alone he introduced over 250 varieties and, of all his fruits, the plums were the greatest contribution, ten of them being standard shipping varieties wherever Japanese plums are grown. "In California they form the basis of a huge industry."

As for science in the strict sense, that domain of *discovery* and *interchange* and *codification* of knowledge, the author makes it clear that Burbank simply never entered, although he yearned for recognition. The exigencies of circumstance from youth onward forced him into the field of commercial competition and advertising. Could he have come under the influence of Asa Gray or Agassiz he would probably have become a true scientist. As it turned out, he did not believe in Mendelism, although his mendacious promoters tried to show that Burbank discovered the Mendelian laws before the announcement of their rediscovery in 1900 (pp. 369, 378); he was ignorant of the biological significance of mutations (p. 370) and the inheritance of acquired characters (p. 383); and he posed as capable of criticizing scientific theories of evolution. But, had he become a scientist, it is doubtful whether many of his contributions to horticulture would have materialized.

Burbank was both philanthropically and eugenically minded. He gave generously in support of community interests and he was a friend to children. It seems probable that his publications on human betterment helped to pave the way for the initiation of sterilization of feeble-minded in California.

The chapter on Burbank's place in the hall of fame impresses this reviewer as especially good and a brief quotation seems to be justified:

> Burbank's popularity was not due to any particular classification or designation that might be applied to him. The great mass of people do not think of him as a botanist, geneticist, plant breeder, or hybridist; horticulturist, gardener, or naturalist; but as a person who struck out alone into the wilderness of Nature and with his own hands so manipulated plant life—just how they don't know or greatly care—as to produce or "create" a multitude of new forms, some of them useful, others merely beautiful, and that altogether he led a busy, productive, exciting life and by his unselfish labors made the world a better place in which to live. . . . The public is

willing to overlook much in its heroes if their fame has a sound basis. Eccentricities, egotism, and even moral lapses are tolerated. The public cannot love a hero who is a rake in private life but can admire even a Byronesque character if he has real accomplishment to his credit. Burbank was both loved and admired. . . . For these concrete accomplishments and because he was the ferment that stirred others to advance the science of breeding, an intangible achievement, to be sure, but his greatest from the viewpoint of science, he is definitely entitled to a place in the hall of fame; but, all factors duly considered, the best that I can do is to assign him a front seat among the minor prophets, a front seat because of the unusual fact that here is one prophet, at least, who was not without honor among his home people.

There are a few points of adverse criticism. On p. 459 the following statement is made: "Geneticists, a small but influential group of research men in our colleges and higher institutions, have *always* felt that Burbank was a stultifier of their science and that his bid for fame was both impudent and absurd." The italics are mine and I for one must protest against this sweeping generalization. As *contra* evidence let me cite the paragraphs on Burbank on pp. 297–298 in "Genetics in Relation to Agriculture," ed. 1, 1918, by R. E. Clausen and myself. There attention was called to Burbank's interspecific hybrids and composite hybrids as outstanding reasons for his fame, with the concluding sentence; "His aim has always been the tangible result rather than the advancement of scientific knowledge," a generalization with which our author will surely agree.

On pp. 353 and 369 the date of the rediscovery of Mendel's paper on heredity is inadvertently given as 1901 instead of 1900. A more serious slip occurs in the following passage (p. 369): "He (Darwin) also pointed out the how and why of variation through cross pollination, and the possibilities for improvement through natural selection, but it remained for Burbank, alone, undirected and unaided, to demonstrate these truths on a scale so grand and complete that it has been the marvel of the age." As far as I am aware, Burbank produced no evidence whatever bearing upon the role of *natural* selection either in plant breeding or evolution. His constant practise in-

volved the most severe *artificial* selection. The author's statement, therefore, is somewhat misleading to the non-biological reader. Finally, exception must be taken to the implications of the author's comparison of Burbank with Thomas Andrew Knight and Benjamin Franklin, both of whom were true scientists.—E. B. Babcock, *University of California, Berkeley, Calif.*

Chromosome Studies on Salmonidae. By Gunnar Svärdson. Kungl. Lantbruksstyrelsen Meddelanden (Reports from the Swedish State Institute of Fresh-Water Fishery Research, Drottingholm), 23, 1945: 1–151, figs. 1–23, pls. 1–4.

Gunnar Svärdson, in a recent paper, reports the results of studies on the chromosomes of all the Swedish Salmonidae. These results will be of great interest to all students of embryology, cytogenetics and speciation. Only the most important can be summarized here:

1. Although spontaneous polyploid embryos are rare, triploids have been found in *Coregonus* and in the hybrid *Salmo salar* × *trutta,* and polyploid cells occur regularly in the embryonic tissue of all species. Experiments made shortly after fertilization with cold shock, as with the Amphibia, throw light on this situation; cold shock paralyzes the spindle, and the resulting embryos may be haploid, diploid, triploid, or multiform aneuploid, depending on the time relations between the shock and the fertilization process.

2. The hybrid *S. salar* × *trutta* is particularly favorable for such experiments, since the parental chromosome sets can be distinguished. In these hybrids it was found that the haploids produced by cold shock are *maternal,* contrary to the hypothesis proposed for Amphibia, while the triploids arise from fertilization of diploid egg nuclei.

3. The gwyniad, *Coregonus lavaretus,* has a fragment chromosome which appears to be a sex-chromosome, the first reported for fishes. Since haploids produced by cold shock in this species may either have the fragment

chromosome or lack it, and since such haploids are presumed, on the evidence from the hybrid salmon, to be maternal, the female gwyniad must be heterogametic.

4. Most interesting of all, perhaps, is the evidence that the salmonids are *old polyploids:* (a) The diploid chromosome numbers in the eight species are found to be 58 (*Oscerus eperlanus*), 60 (*Salmo salar*), 80 (*S. trutta, S. alpinus, Coregonus lavaretus, C. albula*), 84 (*Salmo fontinalis*) and 102 (*Thymallus thymallus*). These numbers can be written $6x-2$, $6x$, $8x$, $8x+4$, and $10x+2$. (b) The evidence of internal balance also points in the same direction; in several species where mitotic irregularities are observed there is a tendency for nuclei to have 20, 40, 60, 80 or 100 chromosomes. The lowest chromosome number observed to undergo normal mitosis was 4, in *C. lavaretus.* This evidence indicates either that each chromosome contains a set of mitotic genes, which appears unlikely, or that the present chromosome numbers are multiples of a lower basic number.

5. The author argues convincingly and at length, not only for his interpretation of the results as due to polyploidy in salmonids, but for the view that polyploidy is not so rare in the animal kingdom as Muller's famous rules would imply. It is claimed that two of Muller's objections do not apply to fishes, since their sex-chromosome mechanism is of a different nature from that of *Drosophila.* The third difficulty, that a polyploid animal must find a polyploid mate, was probably overcome during the Pleistocene, when climatic conditions permitted the formation of hybrid swarms of salmonids, with a high number of unreduced gametes. Vandel's alternative hypothesis, of simultaneous fragmentation of a whole chromosome set, is rejected as highly improbable both statistically and cytologically.—Edward S. Deevey, Jr., *Osborn Zoological Laboratory, Yale University, New Haven, Conn.*

SHORTER ARTICLES AND DISCUSSION

EFFECTIVENESS OF INTRASPECIFIC AND INTERSPECIFIC MATINGS IN *DROSOPHILA PSEUDOOBSCURA* AND *DROSOPHILA PERSIMILIS*

When the distribution areas of two or more sexually reproducing species overlap, the maintenance of these species as separate entities is possible only because the gene exchange between them is limited or prevented entirely. Species are reproductively isolated. Any one or a combination of several causes known as reproductive isolating mechanisms may engender isolation between species. Individuals of different species may seldom or never meet because they occur in different habitats within the same geographic area (ecological isolation). Mating or flowering periods of species may fall on different seasons (seasonal isolation). If sexually mature individuals of different species do meet, hybridization may still not take place. Behavior, courtship, and mating habits of different species may be distinct enough to make copulation or sperm delivery difficult or impossible (sexual isolation). Structural dissimilarities in the genitalia, especially in the chitinous copulatory organs of insects, or in the floral parts of plants, may make normal insemination or pollination impossible (mechanical isolation). Spermatozoa of one species may be inviable or poorly viable in the body of another species, or spermatozoa may fail to be attracted to the eggs of a foreign species (gametic isolation); in plants, pollen tubes may fail to grow in the style of another species (gametophytic isolation). If hybrid zygotes are produced, the hybrid organism may degenerate or die at any stage before reaching the reproductive age (hybrid inviability), or the hybrid that reaches the reproductive stage may fail to produce functional sex cells (hybrid sterility). Chromosome and gene recombination in a hybrid may make its progeny (F_2 generation or back crosses) weak or inviable (hybrid breakdown); hybrid breakdown may be brought about also by disturbances in the cytoplasm of the eggs of a hybrid female produced by hybrid chromosomes.

Every one of the above isolating mechanisms may be partial, acting as an impediment to gene exchange without eliminating it altogether, or it may be complete. It is remarkable that, in nature, complete reproductive isolation of species is very often

attained by means of a combination of several isolating mechanisms all of which produce only a partial isolation when taken separately. Such species may often be hybridized quite successfully in experiments, and gene exchange between them may be obtained in laboratories or in experimental gardens. This is one of the sources of the confusion which still persists among biologists concerning the relations of reproductive isolation to speciation. A system of isolating mechanisms that is completely effective in nature may sometimes be disrupted in experiments.

Drosophila pseudoobscura Frolowa and *Drosophila persimilis* Dobzhansky & Epling (the latter formerly known under the misleading name of "race B") are an example of a pair of species which apparently do not exchange genes in nature, but which can be induced to do so in the laboratory (Dobzhansky and Epling 1944). Although *D. persimilis* shows a preference for cooler and more humid regions than *D. pseudoobscura,* their distribution regions overlap, and in a considerable territory they live side by side. There is a pronounced, but incomplete, sexual isolation between the two species (Lancefield 1929, Mayr and Dobzhansky 1945). The F_1 hybrids are normally viable, but the F_1 hybrid males are completely sterile. The progenies obtained by back-crossing F_1 hybrid females to males of the parental species show a hybrid breakdown. The hybrid chromosomes influence the cytoplasm of the eggs in such a manner that the individuals that develop from these eggs are more or less weak or inviable. Still another reproductive isolating mechanism between *D. pseudoobscura* and *D. persimilis,* which is by itself so weak that it has been overlooked up to now, is described below.

In the experiments described by Mayr and Dobzhansky (1945), Levene and Dobzhansky (1945), and Mayr (1946a, b), many females of *D. pseudoobscura* and *D. persimilis* inseminated by conspecific or by foreign males were dissected for examination of their sperm receptacles. Dr. E. Mayr has first called the attention of the writer to the fact that the females inseminated by conspecific males seem to have on the average considerably more sperm in their receptacles than do females inseminated by foreign males. The amount of the sperm present in a receptacle varies however greatly from female to female, and it is difficult to measure it with any precision. Counting of individual spermatozoa with the aid of the technique of Kaufmann and Demerec (1942) is too laborious for statistical purposes. Therefore, it

was decided to approach the problem indirectly, by counting the offspring produced by females inseminated by a single copulation with a known male.

Females and males of the two species are aged separately for between one and two weeks at 19° C. Five females and five males of the desired kinds are then placed in each of ten empty glass vials, the transfers being made without etherization. The vials are arranged on a vial rack covered with white paper for good visibility. An observer then watches the behavior of the flies in all the vials. As soon as a copulating pair appears in any vial, it is separated from the other flies. The copulation, whether intraspecific or interspecific one, lasts on the average about 6 minutes at room temperature (23–24° C). When the copulating pair separates, the male partner is killed, and the female transferred without etherization into a regular culture bottle with food medium. Every female is, consequently, known to have copulated once and only once. The bottles with the females are placed in an incubator at 25° C. The females are transferred at two-day intervals into fresh bottles as long as they deposit any fertile eggs. The bottles with the eggs are placed at room temperature to develop. Complete counts of the flies that hatch are made in all bottles.

A summary of the data obtained is shown in Table 1. The column marked "d" shows the number of days during which each female remained fertile (this number is obtained by multiplying the number of fertile transfers by two, since the transfers have been made at two-day intervals). The column "n" records the total number of adult offspring of each female. Between 20 and 26 females have been examined for each of the four possible crosses. Six *persimilis* and four *pseudoobscura* females produced no offspring at all, and are not included in Table 1. The sterility of these females was probably accidental: four of them died in a few days, and the remaining ones deposited only few eggs.

As in the experiments of Kaufmann and Demerec (1942) on *D. melanogaster,* the fertility of the females in our material was highest immediately after the copulation or in a few days thereafter, and then declined more or less rapidly. In at least a majority of the females, the decline was obviously due to exhaustion of the sperm supply and not to any loss of the activity of the ovaries; indeed, most females continued to lay many eggs which gave rise to only few larvae, and eventually to no larvae at all.

TABLE 1

NUMBER OF DAYS DURING WHICH THE FEMALE WAS DEPOSITING FERTILE EGGS (d) AND NUMBER OF OFFSPRING PRODUCED (n) AFTER A SINGLE COPULATION

pseudoobscura♀ × pseudoobscura♂		persimilis♀ × pseudoobscura♂		pseudoobscura♀ × persimilis♂		persimilis♀ × persimilis♂	
d	n	d	n	d	n	d	n
4	52	4	42	10	134	18	207
36	454	10	111	6	130	2	128
24	494	12	136	28	607	12	193
20	385	8	56	8	111	12	218
16	383	12	69	14	112	8	232
24	411	2	3	14	180	18	411
22	375	4	103	16	185	8	132
10	194	6	45	26	254	18	387
8	29	2	44	4	91	10	115
8	222	8	85	2	6	10	333
6	175	6	300	10	413	4	39
4	114	4	88	6	98	16	315
10	189	4	161	2	3	6	218
6	125	4	113	6	66	12	356
10	372	4	69	8	191	8	348
4	122	4	100	6	103	4	159
8	240	4	67	8	223	12	178
10	529	4	112	4	190	12	498
10	327	4	98	2	2	6	188
12	199	6	101	6	118	4	105
		4	78	8	115	8	287
		6	109	8	144	6	128
		6	122	10	118		
		4	134				
		4	120				
		4	121				

The data in Table 1 show that the fertility of a female, inseminated either by a male of its own or of a foreign species, is highly variable. One *pseudoobscura* female inseminated by a *pseudoobscura* male remained fertile in 18 successive transfers, i.e. for 35–36 days, and produced a total of 454 offspring. The maximum number of offspring, 607, happens to have been produced by a *pseudoobscura* female inseminated by a *persimilis* male. On the other extreme, several females deposited fertile eggs only in the first transfer, and produced progenies of a few flies only. These variations may be due to fluctuations in the numbers of the eggs matured in the ovary of a female, or to fluctuations in the numbers of spermatozoa received during the copulation. The first of these variables is probably more important than the second. The averages for the different crosses are shown in Table 2.

TABLE 2

MEAN NUMBERS OF DAYS DURING WHICH FERTILE EGGS WERE LAID AND MEAN NUMBERS OF OFFSPRING PRODUCED BY A SINGLE FEMALE

	pseudoobscura♀ × pseudoobscura♂	persimilis♀ × pseudoobscura♂	pseudoobscura♀ × persimilis♂	persimilis♀ × persimilis♂
Days fertile	12.6 ± 1.9	5.4 ± 0.5	9.2 ± 1.4	9.7 ± 1.0
Number of offspring	269.5 ± 33.4	99.5 ± 10.6	156.3 ± 27.6	235.2 ± 24.9

It can be seen that *D. pseudoobscura* females inseminated by males of their own species remain fertile about as long as they do after insemination by *persimilis* males (12.6 and 9.2 days respectively, the difference probably not significant). Insemination by conspecific males produces, however, slightly more progeny than does insemination by foreign males (the difference is 113.2 ± 43.3 individuals, which is most likely significant). *D. persimilis* females inseminated by *persimilis* males deposit fertile eggs for 4.2 ± 1.1 days longer than do the same females inseminated by *pseudoobscura* males. The greatest difference is, however, in the fertility: a *persimilis* female inseminated by a conspecific male produces a progeny about twice as large as it does when inseminated by *pseudoobscura* male (the difference, 135.7 ± 27.1 individuals, is clearly significant).

Several possibilities must be considered in interpreting the differences observed. The progenies of females inseminated by males of a foreign species consist of interspecific hybrids; if hybrids are constitutionally weaker than representatives of the parental species, the hybrids reaching the adult stage may be less numerous than the non-hybrids. This explanation is, however, of doubtful value when applied to the F_1 hybrids of *D. persimilis* and *D. pseudoobscura*, because these hybrids (in contrast to the offspring of the backcrosses) appear to be at least as vigorous as either pure *D. persimilis* or pure *D. pseudoobscura*. Moreover, the lessened viability of the hybrids would not explain the decrease in the interval of time during which the female inseminated by male of a foreign species remains fertile.

Another possibility is that the spermatozoa find a more favorable environment in the sperm receptacles of conspecific than in those of foreign females. A lessened viability of the spermatozoa in foreign sperm receptacles would account for the smaller numbers of hybrids than of the pure species produced in the experiments under discussion. This possibility is rendered plausible by the finding of Patterson, Stone, and Griffen (1942) that the sperm of *D. virilis* survives for only about a day in the seminal receptacles of *D. americana*, while it lives for ten or more days in the receptacles of conspecific females. A similar but less striking reduction of the viability of spermatozoa of *D. americana* in the receptacles of *D. virilis* females is also observed. Oliver and Anderson (1945) found that the sperm stored in the sperm receptacle of females of the mutant glossy in *D. melanogaster* loses

its viability sooner than in the receptacles of wild-type females. Here the action is, however, non-specific, since the sperm viability depends upon the female only, and not upon the male that had produced the spermatozoa.

Although the possibility can not be excluded that a lowered viability of foreign spermatozoa in the seminal receptacles results in smaller progenies from interspecific copulations, there is some evidence against this view. Namely, the fertility of females inseminated by foreign males declines just as gradually as that of females inseminated by conspecific males. A greater and greater proportion of the eggs deposited on successive days are unfertilized, and finally none of the eggs give rise to larvae. Death of the spermatozoa in the unfavorable environment of the foreign sperm receptacles would probably result in a more abrupt termination of the fertility period (see Patterson, Stone and Griffen 1942).

The data presented above agree best with the view that fewer spermatozoa are transferred during interspecific than during intraspecific copulation. This hypothesis was suggested also by microscopic observations on the quantity of the sperm in the sperm receptacles of females inseminated by conspecific and by foreign males (see above). Of course, the number of offspring produced by a Drosophila female is only a fraction of the number of spermatozoa received by that female during copulation (Kaufmann and Demerec 1942). However, the number of spermatozoa received by a female is a limiting factor of its fertility. Just why copulation between *D. pseudoobscura* and *D. persimilis* leads to delivery of fewer spermatozoa than does conspecific copulation is not clear. Once a copula is established, there is no apparent difference between the duration of intraspecific and interspecific copulation. The genitalia of the two species are identical. Mayr (1946b) found that males of either species court females of their own and of the foreign species about equally frequently but interspecific courtship is mostly broken off when the male attempts to mount the female. Despite the morphological identity of the genitalia, interspecific copulation evidently encounters some difficulties. Normal ejaculation may, then, be interfered with during interspecific copulation, and this results in delivery of fewer spermatozoa than are transferred in copulation between individuals of the same species.

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TUMOR FORMATION IN RELATION TO THE ORIGIN OF VIRUSES

A very striking fact in connection with virus infections is that they often cause renewed growth of tissues (neoplastic growths), such as the hyperplasia in fowl pox and the granulations in lymphogranuloma. By contrast, bacterial infections as a rule do not cause neoplastic growths. This would indicate that viruses cause such growths not because of injury in itself, but for other reasons.

It is possible that the neoplastic growths above referred to are bound up with the origin of viruses. According to the viroid theory (Altenburg, 1946), viruses arise from ultra-microscopic symbionts (viroids) universally present in cellular organisms, cancer viruses arising anew as mutants from a viroid in each case of cancer, and ordinarily being non-transmissible because they can not, as a rule, live for even the short period necessary for transmission, apart from the cell to which their viroid parent had become adapted over a long period of evolution. However, very exceptionally, a tumor-inducing virus is transmissible by natural means. This is probably true of a certain virus which causes a tumor in cottontail rabbits (Shope, 1932).

Transmissible tumor-inducing viruses may have played a very important role in the origin (from viroids) of ordinary infectious viruses, such as those that cause yellow fever and smallpox. This

might be expected on the viroid theory. For a viroid, like any other intra-cellular symbiont, could not multiply indefinitely within a non-growing host cell, if for no other reason than that the amount of space within the cell is limited. The materials needed for the growth of the symbiont are also limited. The symbionts must therefore cease multiplying after they have attained the numerical limit that the host cell can harbor. In the case of a green symbiont, such as the *Zoochlorella* in the green Hydra, the number is a few dozen; in *Paramecium bursaria,* a few hundred. The number of viroids would likewise be limited. Now, if one of the viroids in a given cell mutated, and if that cell were already "full," the mutant could not multiply, particularly if it made substantially the same demands on space and materials as the viroid parent, as might often be expected. In a non-multiplying cell, therefore, the new mutant would often remain an isolated individual. Even if it destroyed the host cell and got into a new cell, it would still be incapable of multiplying, provided the new cell did not grow and multiply. It could not, in the case of a virus, multiply in the lymph or blood stream, since viruses can multiply only in living cells. Thus, if a virus arose as a mutant from a viroid in a non-growing cell, it could not as a rule spread.

Conceivably the mutant might sometimes arise in an undifferentiated and growing cell of the embryo, and it might then spread to a larger number of cells in the adult. In growing cells it might even come to outnumber greatly the parent viroid by a faster rate of multiplication. But it would eventually cease to multiply if it ended up in differentiated cells which themselves ceased to multiply. All this is on the assumption that the mutant had substantially the same nutritional requirements as its viroid parent.

But even if the mutant did not have the same requirements as its parent, it is conceivable that it might still require, in common with it, some material present in limited amount in the host cell. This material would act as a limiting factor on the growth of the mutant, and it would tend to prevent the growth of the mutant despite its otherwise different requirements. If, for example, viroids and viruses had lost their capacity to produce a certain fraction of the nucleoprotein molecule, then they would be dependent on the host cell for it. This the host cell would release from its nucleus into its cytoplasm during growth, but not ordi-

narily after it had ceased growing. Unless, therefore, the mutant had acquired the very special capacity to tap the stationary nucleoproteins of the host nucleus for the necessary fraction, and at the same time to utilize it more rapidly than its viroid parent, the nucleoprotein fraction would act as the limiting factor on the multiplication of the mutant.

By contrast, if a viroid gave rise to a mutant which caused the continued multiplication of the cell in which it arose, the mutant itself could continue to multiply and at the same time it might displace the viroid parent in that type of cell if it (the mutant) happened to have a faster rate of multiplication than its viroid parent. In some cases the mutant might get into the blood by the breakdown of some of the tumor cells, and it might then be transmitted to another individual by an insect or other vector, as is probably true in the case of the virus which causes the tumor in cottontail rabbits, above referred to.

If the mutant happened to arise in a type of cell which continued to multiply in the adult (such as one of those at the base of the epidermis), it might multiply and spread to other cells of the same type. But such cells constitute a small fraction of the total in the body; and so, before a mutant could get established, it would as a rule first have to cause tumor-formation, and in addition it would have to be transmissible.

Transmissible tumor-producing viruses undoubtedly form only an exceedingly small fraction of the total new mutants arising from viroids, as above indicated, but as spontaneous tumors are very common in mammals and other vertebrates, the absolute number of these viruses would, over a long period of time, be large. By further mutation, some of these infectious tumor-producing viruses might come to invade types of cells which they did not cause to grow and divide, but which they destroyed or injured, as in the case of yellow fever and the neurotropic virus diseases (rabies, poliomyelitis, etc.). Perhaps this capacity to attack a non-growing cell was dependent upon very special mutations which make possible the destruction of the host-cell nucleus and the release of its nucleoproteins, as suggested, for example, by the ring of chromatin at the margin of the nucleus in cells infected by Virus III of rabbits. The less highly evolved viruses, however, do not have the capacity to attack non-growing cells, as suggested by the fact that the bacterial viruses (presumably rather primitive viruses) do not ordinarily multiply in cells that are

completely non-growing.[1] At the same time that the more highly evolved viruses were acquiring their special capacity for attacking new types of cells, they might, in some cases, have lost their capacity to induce tumor formation in the type of cell originally invaded, and thus they would have become infectious viruses of the ordinary sort (such as the yellow fever virus and the neurotropic viruses). They might also at the same time have evolved very special methods of transmission, as by means of a particular insect or by means of a definite portal of entry, particularly the nose and throat.

In view of the above considerations, it seems likely that few, if any, infectious viruses arose directly from viroids, but rather that they at first caused tumors. This would apply even to mutants which arose in the germ track. For any mutant which injured a germ cell would prevent that cell from reproducing the individual, and so the mutant could not get to a new individual by way of the germ track itself. On the other hand, if the mutant did no injury to the germ cells themselves, but to some other type of tissue, it would probably cause the early death of the offspring, at the time the tissue in question was becoming differentiated, and so the mutant would end its own career. Perhaps it is for this reason that very few viruses are transmitted through the germ track. But if the mutant first caused a tumor of the germ track and was transmissible by an insect (after breaking down the tumor cells and getting into the blood), then it might readily get established. It might then give rise to a typically infectious virus as above described for tumor-inducing mutants in general. In brief, then, *tumor formation would be a possible mechanism by which ordinary infectious viruses arise from viroids.*

A tumor-inducing mutant is, from the standpoint of the tumor cell, useful to that particular type of cell, since it causes that cell to grow and divide; but it is useful to this type to the exclusion of all others. We might call mutants of this type *idio-viroids* (the term "viroid" meaning in general a *useful* ultra-microscopic symbiont). From the standpoint of the organism as a

[1] Ellis and Spizizen (1940) have found that bacterial viruses multiply in the absence of bacterial growth in bacteria (colon bacilli) suspended in dilute solutions of pure glycine. But perhaps the glycine acts in conjunction with materials which were stored up in the bacteria and which were produced during their rapid growth before their transfer to the glycine medium, as suggested by Delbrück (1946).

whole, however, tumor-inducing mutants are injurious and therefore are viruses. As a rule they would be neo-viruses, since they would not be transmissible and therefore they would have to arise anew in each host; but occasionally they would be transmissible (as in the case of the tumor-inducing virus in cottontail rabbits), and therefore they might be paleo-viruses (not newly arisen in the individual with the tumor). Neo-viruses would not necessarily always be tumor-inducing; but unless they were, they would seldom survive, as above pointed out. We can refer to a neo-virus which destroys its host cell immediately (without first causing it to grow and divide) as an *idio-virus*.

The schema shown below, then, gives the classification and evolutionary relationships of viroids and viruses, as above postulated.

Viroids → Neo-viruses
- Idio-viruses (injurious to host cell)
- Idio-viroids (tumor-inducing)
  - Non-transmissible
  - Transmissible → Paleo-viruses

If the above relationships are true, then tumors and infectious virus diseases are intimately connected in point of origin. There is, in fact, no sharp line of demarcation between the two, for there exists every graduation between typical tumors and infectious virus diseases (Andrewes, 1934, Duran-Reynals and Shrigley, 1944). The above theory accounts for this, as well as for the further fact that virus infections so often cause neoplastic growths.

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SPONTANEOUS MUTATION RATE IN DROSOPHILA

THE problem of spontaneous mutation requires for its elucidation additional data concerning the time of occurrence, with respect to the division of the chromosome, of the intrachromosomal readjustments that are reflected in altered genic function. With this purpose in mind, studies of the rate of spontaneous mutation in *Drosophila melanogaster* were undertaken in the summer of 1944. Since there seems little prospect of extending these studies in the immediate future, and since Muller (1946) has reported in an abstract the results of an extensive series of investigations dealing with the same problem, it seems desirable to present at this time, in more complete form than in the preliminary statement (Kaufmann, 1945), the data so far accumulated.

Spermatozoa of Drosophila provide material for measuring the rate of spontaneous mutation in fully-differentiated cells. Mature spermatozoa present in the testes at the time of emergence of the males will be retained if the flies are withheld from copulation. By storing the males and controlling the time of mating, measurements can be made of the effect of ageing of the spermatozoa on mutation rates. However, the interpretation of data so obtained is not unequivocal, since the possibility exists that mitoses occurring in the testes during the period of storage of the males may make available for copulation sperms that were not mature at the time of emergence of the flies. This uncertainty may be avoided if mutation rates can be determined by using spermatozoa that were aged in the seminal receptacles following insemination of females. Experiments utilizing both of these approaches were initiated, but at the time that the work was interrupted the bulk of data accumulated concerned the effects of ageing of cells retained within the adult males.

The frequency of lethal mutation in the X-chromosome of the Swedish-b stock of *D. melanogaster* was determined by the standard ClB method. The Sw-b males were obtained from cultures having their origin in a single pair of flies that had been tested cytologically to insure freedom from chromosomal aberrations. All stocks and experimental materials were kept at 22° C. (a toluene-vapor, mercury thermostat controlled the temperature within the limits of $\pm$ .3° C.). Males were selected within a few hours of their emergence, stored for either one day, 16 days or 32 days, and then placed for one day with virgin females of a g^2 pl/ClB stock. Bar females obtained from these crosses were

mated with males carrying recessive markers in the X-chromosome, so that non-disjunctional male offspring might be detected. All questionable F_2 cultures were checked by additional matings.

Results of the tests are presented in Table 1. The 1–2-day and the 32–33-day samples are significantly different (3.5 times the standard error); together with the 16–17-day material they indicate a relation of mutation to passage of time that is essentially a linear proportionality. These results parallel those obtained by Timoféeff-Ressovsky (1935), who reported that spermatozoa of previously unmated males tested 20 days after emergence of the flies gave a lethal mutation rate of .243 ± .052 in contrast with

TABLE 1

SPONTANEOUS LETHAL MUTATION RATE (CIB TESTS) AMONG SPERMATOZOA OF PREVIOUSLY UNMATED SW-B MALES OF D. MELANOGASTER 1–2 DAYS, 16–17 DAYS, AND 32–33 DAYS AFTER EMERGENCE OF THE FLIES. TEMPERATURE OF STOCKS AND EXPERIMENTAL MATERIAL 22° C.

Days after emergence	Number of		Per cent mutations
	Sperms tested	Lethal mutations	
1–2	3545	5	0.141 ± 0.063
16–17	3471	11	0.317 ± 0.095
32–33	5248	30	0.572 ± 0.104

	Diff. / S.E.	Significance
1–2 cf. with 16–17	.176 / .114	= 1.5
1–2 cf. " 32–33	.431 / .122	= 3.5
16–17 cf. " 32–33	.255 / .141	= 1.8

a percentage of .102 ± .032 among spermatozoa of males tested shortly after emergence.

Very few offspring were obtained from the preliminary set of 22° C. "controls" in which the sperms were stored for 16 days in the seminal receptacles of CIB females that had been inseminated during their second day of adult life. Most of the spermatozoa were utilized by the females in fertilizing eggs laid during the first few days after mating, despite efforts to impose conditions that would inhibit oviposition. However, the sample of about 200 F_2 cultures obtained, which included an X-chromosome lethal, was sufficiently large to indicate that the method can be adapted to experimental use. Inhibition of oviposition may be assured if the females are stored at low temperatures (Muller, 1940), but under such conditions the percentage of spontaneous mutations may be expected to be considerably lower than at 22 degrees (Muller and Altenburg, 1919; Muller, 1927; 1928; Timoféeff-Ressovsky, 1935).

The data obtained by testing mature spermatozoa suggest that the mutations accumulate in nondividing chromosomes. In con-

firmation of this interpretation, Muller (1946) reports that the mutation frequency of spermatozoa stored in the female also rises considerably. Accumulation of gene mutations with increasing age has been reported for seeds and pollen of Datura (Cartledge and Blakeslee, 1933, 1934, 1935; Cartledge, Murray and Blakeslee, 1935), and for seeds of Antirrhinum (Stubbe, 1935, 1936). An increase in the frequency of spontaneous chromosomal aberrations with increasing age has also been found to occur in the tissues of resting embryos of seeds (Navaschin, 1933; Gerassimova, 1935; Navaschin and Gerassimova, 1936; Nichols, 1941). In some organisms the mutation process appears to be related to the mitotic cycle. Experiments on bacteria, for example, have indicated that the proportion of mutated to nonmutated types increased mostly or exclusively during the growth phase of the culture (Luria and Delbrück, 1943; Zamenhof, 1945). In experiments in which mutants seem to appear after a culture has reached full growth, there is no conclusive evidence that some cell division has not taken place. Some of the data obtained from Drosophila, as in Demerec's studies of mutable genes (1928, 1929), suggest that the mutations occur at the time of the division of the cells. The possibility that the conditions facilitating spontaneous mutations may vary in different types of cells has recently been demonstrated by Muller (1946).

Alterations in the structural pattern of the fundamental genic materials that result in the accumulation of mutant loci in nondividing cells might be referable to thermal agitation and related phenomena, as Timoféeff-Ressovsky, Zimmer and Delbrück (1935) have visualized. Perhaps a state of dynamic equilibrium may exist even in these complex systems in which portions of the nucleoprotein molecules essential to gene structure or function are constantly being replaced in the living cell (Marshak and Walker, 1945). Under such conditions displacement of one unit by another of different structure might so alter the functional capacity of the gene that the process of normal embryonic development would be disturbed.

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Summary

The frequency of spontaneously occurring lethal mutations in the X-chromosome of the Sw-b stock of *D. melanogaster* was found to be higher among spermatozoa of previously unmated

males 16 to 17 days, and 32 to 33 days after emergence than among 1- to 2-day old males (Table 1).

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